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Page 108, line 22, "between 0.05 and 0.0100 per cent" should read "between 0.05 and 0.100 per cent."

Page 217, heading, "The Citrus-Root Nematode Tylenchus Semipenetrans" should read "The Citrus-Root Nematode Tylenchulus Semipenetrans."

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# FLAVOR OF ROQUEFORT CHEESE

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## INTRODUCTION

Every cheese connoisseur is familiar with the peculiar peppery or burning effect of well-ripened Roquefort cheese on the organs of taste. This effect is so characteristic of this variety of cheese that its quality is commonly expressed in terms of its "hotness." The purpose of this investigation was to identify and to explain the occurrence in the cheese of any substances which contribute to this particular flavor.

## WORK OF OTHER INVESTIGATORS

Weigmann (16, p. 187)[2], without citing the source of his information, says:

> It has long been known that the characteristic rancid, sharp taste of French Roquefort, English Stilton, and Italian Gorgonzola cheeses is caused by the green Penicillium. This characteristic taste is first observed after the spores and spore bearers have formed and has therefore been ascribed to them.

If a chemical substance which imparts to the cheese this burning taste were elaborated in the spores and spore bearers, the mycelium of the mold grown upon artificial media should show this taste in a marked degree at the spore-bearing stage. No taste, however, can be detected at any stage, except a slight bitterness.

Jensen [8] concluded that the chief constituent of the aroma of Roquefort cheese was the "very sharp-tasting ethyl butyrate," but offered no data to show that this ester was actually present. He apparently identified it only by the odor.

Esters are so commonly known as the flavoring substances of fruits that it is quite natural to ascribe to them a part in the production of flavor in other foodstuffs. In fact, Suzuki, Hastings, and Hart (13, p.

[1] The writer takes pleasure in acknowledging his indebtedness to Dr. Charles Thom, who has kindly provided him with all the pure cultures of *Penicillium roqueforti* used in this investigation.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 13-14.

456), found small quantities of alcohols and esters in the neutral "flavor solution" obtained from Cheddar cheese.

The neutral flavor solution from 750 grams of a ripe Roquefort cheese was studied, using the method outlined by the above-mentioned investigators. The acids in ester combination in this large mass of cheese totaled only 0.44 decinormal c. c. It is hardly possible to identify accurately the acids of a mixture so small in quantity. The proportional numbers obtained by a Duclaux distillation approximated the constants for acetic acid. The odor of Roquefort cheese suggests ethyl acetate, and this ester may be partially responsible for the typical aroma, but it is doubtful whether the quantity is large enough to materially influence the taste.

As early as 1877, Nencki (10, p. 1033), attempted to isolate the substance giving to Roquefort cheese its piquant taste. He acidified about one pound of cheese with sulphuric acid and distilled it with steam. After filtering the distillate he neutralized it with sodium hydroxid and extracted it with ether. After evaporation the ether extract left a "very volatile, slightly yellow-colored oil of sharp burning taste, neutral reaction, and characteristic moldy odor." The quantity of this oil was not sufficient for identification.

Repeated attempts with the ripest cheese procurable were made to duplicate the results of Nencki. Very small quantities of a gummy white mass were obtained, which consisted chiefly of the sodium salts of butyric and caproic acids. Although the oil described by Nencki was not found by following his procedure, it was observed that globules of an insoluble oil floated upon the surface of the distillate. This oil, which Nencki apparently filtered off and discarded, was identified as a mixture of the volatile and soluble acids of milk fat. The amount of these insoluble acids increased with the ripeness of the cheese distilled and appeared to be a normal product of the curing process. Since these acids possessed a peppery taste and the cheese mass after distillation was almost tasteless, a detailed study of their origin and relation to the flavor of the cheese was made.

## EXPERIMENTAL METHODS EMPLOYED

Typical Roquefort cheeses of various stages of ripeness were purchased in the retail markets and used in these experiments. In general, the method of study outlined by Jensen was followed.

After scraping off any slime on the surface of the cheese, a wedge-shaped section extending from the periphery to the center was cut out, minced, and thoroughly mixed. A sample of 50 to 150 grams, depending on the ripeness of the cheese, was weighed, rubbed to a smooth cream with warm water in a mortar, and rinsed into a 500 c. c. Kjeldahl flask. The suspension was made up to a volume of 250 c. c. of dilute sulphuric acid added in slight excess, as indicated by a blue color with Congo red, and

distilled with steam until 1,000 c. c. of distillate passed over. During the distillation a small flame was kept under the Kjeldahl flask and regulated so that the volume of the suspension remained nearly constant.

The fatty acids which separated from the distillate were filtered off, dissolved in alcohol, titrated with decinormal barium hydroxid, and reported as insoluble acids.

The filtrate was neutralized with decinormal barium hydroxid and evaporated to about 100 c. c. This solution was then partially decomposed with normal sulphuric acid and the liberated acids distilled out. The distillate was designated "Fraction I." The residue was again made up to 100 c. c., partially decomposed with normal sulphuric acid, and distilled, giving Fraction II. By repeating this process of partial decomposition followed by distillation the soluble acid portion was divided into several fractions containing about equivalent quantities of acid. The acids of greater molecular weight occur in the first fractions because of their weaker chemical affinity and more rapid rate of volatilization.

## A QUALITATIVE STUDY OF THE VOLATILE ACIDS

The distillate of a well-ripened cheese was divided into five fractions. Fraction I consisted of the insoluble acids adhering to the condenser after rinsing with cold water. The remainder of the insoluble acids constituted Fraction II. The soluble acids were divided into Fractions III, IV, and V by the process of partial decomposition of the barium salts with normal sulphuric acid followed by distillation, as previously described.

The barium salts were prepared by titrating with decinormal barium hydroxid, dried to constant weight at 120° C., moistened with dilute sulphuric acid, ignited, and weighed as barium sulphate. The results are detailed in Table I.

TABLE I.—*The barium salts of the volatile acids of Roquefort cheese.*

| Fraction No. | Decinormal equivalent. | Percentage of barium sulphate found from barium salts. | | |
|---|---|---|---|---|
| | | Salt. | Actual. | Theoretical. |
| | *C. c.* | | *Per cent.* | *Per cent.* |
| I | 2.25 | Barium caprate..... | 45.53 | 48.64 |
| II | 5.80 | Barium caprylate... | 47.18 | 55.08 |
| III | 17.32 | Barium caproate.... | 64.67 | 63.48 |
| IV | 17.36 | Barium butyrate.... | 76.07 | 74.91 |
| V | 18.58 | Barium acetate..... | 88.65 | 91.37 |

A study of the above results shows that the distillate from this variety of cheese is essentially a mixture of the volatile acids of milk fat. Capric acid is the chief constituent of the insoluble-acid portion. The fact that no fraction shows the presence of an acid of a molecular weight between capric and caproic indicates that the quantity of caprylic acid is small.

## QUANTITATIVE ESTIMATION OF THE VOLATILE ACIDS

The Duclaux method of fractional distillation was employed for the quantitative estimation of the volatile acids.

In order to illustrate fully this method, complete data for one cheese will be given. The soluble-acid portion of the distillate from 150 grams of this cheese required for neutralization 57.20 c. c. of decinormal barium hydroxid, and the insoluble-acid portion, 12.15 c. c.

The soluble-acid portion was divided into four fractions, which were distilled in the order of numbering. The remaining 10 c. c. of one fraction was always added to the succeeding fraction before making up to 110 c. c. Therefore, the only acids not taken into account would be in the last 10 c. c. of the final fraction. These can be readily calculated, since the 100 c. c. of distillate collected contained all of the caproic, 97.5 per cent of the butyric, and 80 per cent of the acetic acid.

The data for the analysis of the soluble-acid portion are given in Table II. A represents the titer of each successive 10 c. c. portion of the distillate; B, the sum of these titers; C, the percentage of B in terms of the acidity of 100 c. c. of distillate, and D, the corresponding figures calculated from the Duclaux constants for the combination of acids indicated.

TABLE II.—*Data for the quantitative estimation of the soluble acids in Roquefort cheese.*

FRACTION I.[1]

| Item. | 10 c. c. | 20 c. c. | 30 c. c. | 40 c. c. | 50 c. c. | 60 c. c. | 70 c. c. | 80 c. c. | 90 c. c. | 100 c. c. |
|---|---|---|---|---|---|---|---|---|---|---|
| A.... | 3.01 | 2.29 | 1.71 | 1.04 | 0.71 | 0.49 | 0.34 | 0.27 | 0.18 | 0.14 |
| B.... | 3.01 | 5.30 | 7.01 | 8.05 | 8.76 | 9.25 | 9.59 | 9.86 | 10.04 | 10.18 |
| C.... | 29.57 | 52.06 | 68.86 | 79.08 | 86.05 | 90.86 | 94.20 | 96.86 | 98.62 | 100.00 |
| D.... | 29.05 | 49.73 | 67.66 | 78.72 | 86.37 | 91.64 | 94.42 | 96.75 | 98.76 | 100.00 |

FRACTION II.[2]

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A.... | 3.10 | 2.20 | 1.64 | 1.12 | .85 | .65 | .47 | .36 | .27 | .19 |
| B.... | 3.10 | 5.30 | 6.94 | 8.06 | 8.91 | 9.56 | 10.03 | 10.39 | 10.66 | 10.85 |
| C.... | 28.57 | 48.85 | 63.96 | 74.29 | 82.12 | 88.11 | 92.44 | 95.76 | 98.25 | 100.00 |
| D.... | 26.18 | 45.70 | 62.62 | 74.04 | 82.44 | 88.68 | 92.44 | 95.69 | 98.24 | 100.00 |

FRACTION III.[3]

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A.... | 2.88 | 2.22 | 1.71 | 1.32 | 1.02 | .82 | .65 | .51 | .37 | .30 |
| B.... | 2.88 | 5.10 | 6.81 | 8.13 | 9.15 | 9.97 | 10.62 | 11.13 | 11.50 | 11.80 |
| C.... | 24.41 | 43.22 | 57.71 | 68.90 | 77.54 | 84.49 | 90.00 | 94.32 | 97.45 | 100.00 |
| D.... | 23.45 | 41.64 | 57.49 | 69.00 | 77.87 | 84.85 | 89.61 | 93.83 | 97.25 | 100.00 |

[1] 72 parts of caproic and 28 parts of butyric acid; 7.33 c. c. of caproic and 2.85 c. c. of butyric acid.

[2] 54 parts of caproic and 46 parts butyric acid; 5.86 c. c. caproic and 4.99 c. c. butyric acid.

[3] 40 parts caproic, 55 parts of butyric, and 5 parts of acetic acid; 4.72 c. c. of caproic, 6.49 c. c. butyric, 0.59 c. c. acetic acid.

TABLE II.—*Data for the quantitative estimation of the soluble acids in Roquefort cheese*—Continued.

FRACTION IV.[1]

| Item. | 10 c. c. | 20 c. c. | 30 c. c. | 40 c. c. | 50 c. c. | 60 c. c. | 70 c. c. | 80 c. c. | 90 c. c. | 100 c. c. |
|---|---|---|---|---|---|---|---|---|---|---|
| A.... | 3.56 | 3.07 | 2.67 | 2.32 | 2.10 | 1.79 | 1.59 | 1.43 | 1.31 | 1.20 |
| B.... | 3.56 | 6.63 | 9.30 | 11.62 | 13.72 | 15.51 | 17.10 | 18.53 | 19.84 | 21.04 |
| C.... | 16.92 | 31.51 | 44.20 | 55.23 | 65.21 | 73.71 | 81.46 | 88.07 | 94.29 | 100.00 |
| D.... | 16.75 | 31.43 | 44.52 | 55.88 | 65.67 | 74.24 | 81.38 | 88.15 | 94.21 | 100.00 |

[1] 10 parts of caproic, 66 parts of butyric, and 24 parts of acetic acid; 2.10 c. c. of caproic, 13.89 c. c. of butyric, and 5.05 c. c. of acetic acid.

Calculated for 110 c. c.: 2.10 c. c. of caproic, 14.25 c. c. of butyric, and 6.31 c. c. of acetic acid.

Totals for 150 grams of cheese: 20.01 c. c. of caproic, 28.58 c. c. of butyric, and 6.90 c. c. of acetic acid.

Calculated for 100 grams of cheese: 13.34 c. c. of caproic, 19.05 c. c. of butyric, and 4.60 c. c. of acetic acid.

Complete analyses were not made of all the cheeses investigated, and for uniformity all results have been calculated to 100 grams of cheese. Roquefort cheese is very uniform in composition, and the significance of the results would in nowise be altered by calculating to dry matter. Dox (5, p. 239) made careful analyses of eight brands of Roquefort cheese. His results are summarized in Table III.

TABLE III.—*Summary of the analyses of eight brands of Roquefort cheese.*

| Degree. | Water. | Fat. | Protein. | Ash. | Salt. |
|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Maximum | 40.10 | 33.53 | 23.25 | 6.81 | 4.50 |
| Minimum | 37.49 | 31.50 | 19.94 | 5.48 | 3.64 |
| Average | 38.61 | 32.24 | 21.62 | 6.19 | 4.18 |

It is to be noted that the sum of the acids recovered by distillation is only 55.49 c. c., or 1.71 c. c. less than the original acidity. A loss of about this magnitude was always experienced. Part of this loss may be due to carbon dioxid in the original distillate which is not completely redissolved in the process of repeated distillation; another part of it is probably due to decomposition by heat, as observed by Browne (3, p. 819). There is also a slight loss of acid during evaporation, for the salts of the weak organic acids are appreciably hydrolyzed, and an odor of the acids can be readily detected above a hot solution neutral to phenolphthalein.

Inspection of these data indicates that there was probably a small quantity of caprylic acid in the first fractions and a small amount of formic acid in the last fraction. These acids, if present at all, were in too small quantities to be determined accurately by this method and were not entered in the calculations.

The foregoing results are regarded as typical of a well-ripened Roquefort cheese. It is to be expected that a mild cheese will have a lower volatile acid number and a very ripe cheese a higher one. Analyses of the acid distillates of cheeses of differing degrees of ripeness have been made and are summarized in Table IV.

TABLE IV.—*Volatile acids in 100 grams of cheese.*

[Acidity in decinormal c. c.]

| No. | Condition of cheese. | Total volatile acids. | Insoluble acids. | Soluble acids. | Caproic acid. | Butyric acid. | Acetic acid. | Acid number for 10 grams of fat. |
|---|---|---|---|---|---|---|---|---|
| 1 | Slightly ripened..... | 15.07 | 2.90 | 12.17 | 6.85 | 4.14 | 1.18 | ..... |
| 2 | Well ripened... | 45.09 | 8.10 | 36.99 | 13.34 | 19.05 | 4.60 | 56.4 |
| 3 | Overripened.... | 102.23 | 29.30 | 72.93 | 30.38 | 36.30 | 6.25 | 152.6 |

For the determination of the insoluble-acid number of 10 grams of fat reported in the above table, the procedure of Schmid-Bondzynski [1] was followed. The cheese was digested in warm hydrochloric acid of a specific gravity of 1.125 until the fat separated. It was then washed free from hydrochloric acid, was dried and filtered clear. A sample of the fat was weighed out, dissolved in 95 per cent alcohol, and titrated with decinormal sodium hydroxid. The result was calculated to 10 grams of fat.

In order to follow up the increase in acidity of the distillate during progressive ripening, about 1 pound of cheese was minced and placed in a large bottle. A 50-gram sample was immediately weighed out and distilled. The cheese was kept at about 23° C., and on the dates given in Table V the mass was thoroughly mixed and other 50-gram samples were taken and distilled. During this time the cheese developed no abnormal flavors.

TABLE V.—*Volatile acids in 100 grams of cheese.*

[Acidity in decinormal c. c.]

| Date. | Total volatile acids. | Insoluble acids. | Soluble acids. | Caproic acid. | Butyric acid. | Acetic acid. |
|---|---|---|---|---|---|---|
| Mar. 16..... | 44.52 | 8.64 | 35.88 | 12.16 | 21.56 | 2.16 |
| Mar. 18..... | 50.02 | 11.30 | 38.72 | 12.96 | 21.12 | 4.64 |
| Mar. 22..... | 79.98 | 22.20 | 57.78 | 20.04 | 32.64 | 5.10 |
| Apr. 1..... | 122.60 | 33.20 | 89.40 | 38.88 | 46.20 | 4.52 |

[1] See Barthel, Chr. (2, p. 187).

From the data in Tables IV and V and from observations on numerous other cheeses, which have been distilled without making complete analyses of the distillates, we conclude that a well-flavored Roquefort cheese will have a distillation number (c. c. of decinormal alkali to neutralize the distillate from 100 grams of cheese) of 30 to 60; one showing a slight growth of mold and very little flavor will have a distillation number less than 30; and a cheese thoroughly permeated with mold and highly flavored will have a distillation number above 60, and in extreme cases this number may even exceed 100.

## RELATION OF THE VOLATILE ACIDS TO FLAVOR

In discussions of the properties of this homologous series of saturated fatty acids, a fact of very peculiar interest is commonly overlooked—namely, the pronounced changes in taste with increasing molecular weight. The lower members are distinctly sour and in dilute solutions can not be distinguished from the mineral acids by their taste. This sourness diminishes with increasing carbon content and can not be detected at all in those containing more than 7 carbon atoms. Valeric acid, with 5 carbon atoms, has not only a sour taste but also a distinct peppery effect. This peppery effect increases with increasing molecular weight and is very pronounced in caprylic and capric acids. The higher acids of this series, containing 12 or more carbon atoms, have no well-defined taste. Thus, it is seen that the group of acids containing from 5 to 10 carbon atoms, which includes the three volatile acids of milk fat—caproic, caprylic, and capric—is characterized by this burning or peppery effect on the organs of taste.

Volatile acids have been vaguely connected with the aroma and flavor of dairy products by numerous investigators, but to our knowledge the peculiar peppery effect of the green-mold cheeses has never been specifically attributed to this group of volatile, difficultly soluble acids of milk fat. Duclaux (7, p. 288), in a discussion of Cantal cheese, states that "The sharp taste of old cheese is in large part due to the fixed fatty acids and their salts, which have a burning effect on the palate and tongue." Just what Duclaux meant by "fixed" acids is not quite plain, but certainly his observation is applicable only to the volatile acids. We believe that the unmistakable similarity between the effect on the gustatory nerves of well-ripened Roquefort cheese and these volatile acids entirely justifies the conclusion that the peppery taste of the cheese is due to the accumulation of these acids and their readily hydrolyzable salts.

This explanation is not offered as a solution of the entire problem of the flavor of Roquefort cheese. This is rather to be interpreted as an endeavor to identify a single prominent component of the flavor and to explain its occurrence in the cheese.

FORM IN WHICH VOLATILE ACIDS OCCUR

Another point concerning the relation of these acids to the flavor of cheese remains to be considered—that is, the form in which they are combined. It is not to be expected that the acids are all in the free state, for the proteolysis taking place during the ripening process gives rise to ammonia and possibly other basic substances. The exact course of this proteolysis has not been determined. In a ripe Roquefort cheese examined by Jensen 52.50 per cent of the total nitrogen was water-soluble, 23.64 per cent was precipitated by phosphotungstic acid, and 4.99 per cent was in the form of ammonia. This is in accord with unpublished data of Dox,[1] who made a separation of the different classes of nitrogenous products by the method of Van Slyke and Hart (15, p. 150). For a prime Roquefort he gives the distribution of nitrogen as follows: In caseoses, 10.7; in peptones, 8.6; in amino acids, 29.1; in ammonia, 6.1; insoluble, 45.5. Dox (6, p. 423) has, furthermore, identified tyrosin among the amino acids. The work of these investigators indicates that probably all of the hydrolytic cleavage products of paracasein are to be expected in a ripe Roquefort cheese.

Every Roquefort cheese examined in the laboratory of the Dairy Division has been decidedly acid to both litmus and phenolphthalein. The exact amount of acidity is difficult to measure, for such a complex mixture of weak acids and bases can not be accurately titrated. Ten grams of a well-ripened cheese having a distillation number of 43.9 were extracted three times with 50 c. c. of 95 per cent alcohol. This alcoholic extract required 57.0 c. c. of decinormal alkali for neutralization. Data given by Jensen show that the ammonia is not equivalent to the acidity calculated from the distillation number and the acid number of the fat. This does not take into account the acidity of the paracasein and amino acids.

It is also to be remembered that the ammonium salts of the weak organic acids are so strongly hydrolyzed in an aqueous solution that they can not be crystallized from this solvent. However, they can be readily prepared by passing dry ammonia into a benzene solution of the acids. Ammonium caproate, caprylate, and caprate have been prepared in this manner. They are white needlelike crystals which readily give off the odor of the respective acids when exposed to the moisture of the air. The peppery effect of these salts when placed upon the tongue is quite similar to the effect of the free acids, but is less intense.

From these considerations of the basic and acid substances of the cheese it is apparent that caproic, caprylic, and capric acids exist, both free and combined, and those combined are in such weak form of combination that their characteristic taste is not obscured.

[1] Dox, A. W. Records of the Storrs Agricultural Experiment Station.

## ORIGIN OF THE VOLATILE ACIDS OF ROQUEFORT CHEESE

Neuberg and Rosenberg (12, p. 178) have shown that the volatile fatty acids may arise from the putrefaction of casein. From 1 kilogram of casein they isolated 40 grams of butyric acid, 95 grams of caproic acid, 5.0 grams of capric acid, and a considerable quantity of valeric acid. More recently Neuberg (11, p. 501) has shown that putrefactive bacteria produce normal valeric acid from prolin and active valeric acid and active caproic acid from isoleucin. Valeric acid has long been considered as a putrefactive product, and its occurrence in Limburger cheese accounted for in this manner.

The flavor of Roquefort cheese gives no suggestion whatever of the products accompanying putrefaction. Since the volatile acids include those represented in milk fat from lauric to butyric and in about the same proportions as in milk fat, we are led to conclude that they arise from a hydrolysis of the fat.

There is no conclusive evidence that the glycerids of fat are unequally attacked by lipolytic enzyms, and if the volatile acids result from a hydrolysis of the fat the insoluble acid number of the cheese fat should be about proportional to the volatile acid number of the cheese. This is shown to be the case in Table IV.

The biological significance of the small quantity of acetic and possibly formic acid present is not clear. However, these may result from a fermentation of the carbohydrates in the early stages of ripening or may be a product of the partial oxidation of higher acids or glycerin by the mold.

## CULTURAL STUDIES OF PENICILLIUM ROQUEFORTI

No biological study ever reported has shown that any other organism than *Penicillium roqueforti* is essential to the proper ripening of this variety of cheese. Some of the proteolysis is to be attributed to the rennet, as is the case with all renneted cheeses, and the bacterial flora common to milk is responsible for the development of acidity in the curd during the early stages of ripening. The hydrolysis of the fat can hardly be ascribed to either of these agencies. So to obtain more definite information concerning the action of *P. roqueforti* on butter fat, various cultural studies have been made.

Czapek's solution, with such modifications as will be specified, was sterilized with steam and used for all cultures on liquid media.

*Penicillium roqueforti* will grow upon such a solution of inorganic salts as this medium contains when cane sugar is wholly replaced by pure butter fat, tributyrin, ethyl butyrate, glycerin, butyric acid, or ammonium butyrate. Therefore the mold not only has the ability to hydrolyze simple esters and triglycerids but also to utilize their constituents as sources of carbon. This would presuppose the presence of a lipolytic enzym in the organism. Generally, the action of an enzym-containing extract of an organism is so much feebler than the action of the living

organism that the latter method of study has been adopted as affording a truer picture of what the mold actually accomplishes in a ripening cheese.

*Penicillium roqueforti* was grown upon 100 c. c. of Czapek's solution in which cane sugar was replaced by 3 grams of fresh, filtered milk fat. After 50 days at about 23° C. dilute sulphuric acid was added to the culture until a blue color with Congo red was produced. The solids consisting of fat and mold mycelium were filtered off, washed with hot water, dried at 100° C., and extracted over anhydrous copper sulphate with ether in a Soxhlet extractor. The fat of the ether extract was examined and gave the results shown in Table VI.

TABLE VI.—*The effect of the growth of mold on the composition of milk fat.*

| Condition. | Age. | Reichert-Meissl number for 2.5 grams. | Acid number for 10 grams in decinormal c. c. | Molecular weight of insoluble nonvolatile acids. |
|---|---|---|---|---|
| | *Days.* | | | |
| Uninoculated | 50 | 15.87 | 3.20 | 267.6 |
| Inoculated | 50 | [1] 5.66 | 157.40 | 271.7 |

[1] Determination made on 1.8688 grams and calculated for 2.5 grams.

The above data show that the fat of the uninoculated control gave constants typical of normal milk fat and consequently had undergone no changes, while the fat upon which *Penicillium roqueforti* had grown had been about two-thirds hydrolyzed. The filtrate from the culture contained no volatile acids, which would indicate that the soluble acids of the decomposed fat are consumed completely by the mold, while the insoluble acids are much less readily consumed. That other molds attack the fatty acids of low molecular weight more readily than those of higher molecular weight has been demonstrated by Laxa (9, p. 119). This explains the high acid number of the ether extract, for it is evident that if all the acids resulting from the hydrolysis of the fat were consumed, the ether extract of the mold culture would have been nearly neutral and would have shown the Reichert-Meissl number of normal milk fat.

The conditions of food supply maintained in a Roquefort cheese would be more nearly simulated by growing the mold upon fresh curd than upon solids suspended in a liquid medium. Table VI shows the result of the action of the mold on the milk fat in such a culture. To 50 grams of fresh curd containing about 50 per cent of water 2 grams of sodium chlorid were added. (Roquefort cheese contains about 4 to 5 per cent of sodium chlorid.) The curd was sterilized and inoculated with *Penicillium roqueforti* and kept at about 23° C. On the dates designated the fat was separated by the Schmid-Bondzynski method and was examined. See Table VII.

TABLE VII.—*The effect of Penicillium roqueforti on the fat of fresh curd.*

| Condition. | Age. | Reichert-Meissl number for 2.5 grams. | Decinormal acid number for 10 grams. | Molecular weight of the insoluble fatty acids. |
|---|---|---|---|---|
| | *Days.* | | | |
| Uninoculated control | 36 | 16.05 | 2.3 | 269.3 |
| Inoculated | 36 | 12.74 | 66.7 | 271.3 |
| Do | 85 | 7.87 | 163.7 | 269.4 |

As in the previous culture on Czapek's solution, the fat showed decided decomposition, but there was only a meager accumulation of soluble and volatile acids. A culture at the age of 45 days contained only 0.75 decinormal c. c. of soluble acids in 500 c. c. of distillate. A culture similarly grown but in the presence of *Bacillus lactis acidi* contained only 0.80 decinormal c. c. of soluble acids in a like volume of distillate. The acid number of the fat in both cultures showed that more than one-half of it had been hydrolyzed.

Just why these acids accumulate in a cheese but not in a culture grown upon green curd is not obvious. The growth of mold in the crevices of the cheese is always very scant in comparison with the growth of a culture having an unlimited supply of oxygen (Thom and Currie, 14, p. 249). The gas within the cheese frequently contains less than 5 per cent by volume of oxygen. This very limited supply of oxygen may hinder the metabolic functions of the mold and prevent the complete oxidation of butyric and caproic acids.

Another explanation, and to the writer this appears the more probable one, is to be found in the presence of a water-soluble enzym which diffuses beyond the feeding zone of the mold. An examination of a Roquefort cheese will always show that the central portion is much more open in texture and more thoroughly permeated with mold than the outer portion. In fact, a layer about 2 cm. in thickness next to the rind is almost free from mold. This portion, although not so highly flavored as the more moldy portion, is always sufficiently ripened to be entirely palatable. The inner portion of a very ripe cheese gave a distillation number of 88.3 and this outer portion, 60. This thorough ripening in portions of the cheese where only small and scattered pockets of the mold are visible is apparently due to water-soluble enzyms, among which is an active lipase.

## ENZYMOTIC STUDIES

In order to obtain definite proof of the presence of a water-soluble lipase, enzymotic studies were made on the mycelium of *Penicillium roqueforti* grown for six days upon Czapek's solution. Ethyl butyrate to the extent of 0.5 c. c. per 100 c. c. of medium was added. Dox (4, p. 149) has shown that the addition of a particular substrate to a medium

will increase the production of the corresponding enzym. In accordance with this principle, the addition of ethyl butyrate should stimulate the production of lipase.

Preliminary experiments were conducted to discover the most active preparation of enzym. Three methods were tried:

(1) The "acetonedauerhefe" method of Albert, Büchner, and Rapp (1, p. 2376).

(2) Fresh mycelium was triturated with water and powdered glass, filtered, and the filtrate used.

(3) Washed, air-dried mycelium was triturated with dry powdered glass and the pulverized mixture used.

On tributyrin in water, blue with litmus, and with toluene as antiseptic, the "dauer" preparation gave no pink in six days, the water extract showed slight acidity in six days, and the fresh powdered mycelium showed a distinct acidity in 24 hours. Controls of boiled enzym preparations showed no acidity in six days. On ethyl butyrate made distinctly alkaline with one drop of decinormal sodium hydroxid the enzym extract showed acidity in 24 hours and the mycelium powder in 4 hours. Controls showed acidity in five days.

From these preliminary experiments the following conclusions were drawn:

The "dauer" preparation is unreliable for the study of lipase in the mycelium of *Penicillium roqueforti;* a water-soluble lipase can be extracted from the mycelium; and the most active preparation is the fresh pulverized mycelium.

A more accurate measure of the activity of the lipase at 38° C. to 40° C. was made on tributyrin, triacetin, ethyl butyrate, and ethyl acetate. For this study 1 gram of the air-dried mycelium was triturated with 4 grams of powdered glass, and 0.5 gram of the powder was added to 0.5 c. c. of the ester in 50 c. c. of distilled water contained in a small Erlenmeyer flask. The water was covered with a thin layer of toluene. Boiled enzym preparations were used in the controls. The flasks were tightly stoppered. At the end of two weeks the solutions were filtered and titrated with decinormal barium hydroxid. The results are given in Table VIII.

TABLE VIII.—*The action of the lipase of Penicillium roqueforti on esters.*

[Acidity in decinormal c. c.]

| Ester. | Days. | Unboiled enzym. | Control, boiled enzym. |
|---|---|---|---|
| Tributyrin | 14 | 0.67 | 0.08 |
| Triacetin | 14 | .81 | .51 |
| Ethyl butyrate | 14 | 1.37 | .20 |
| Ethyl acetate | 14 | 1.31 | .24 |

These enzymotic studies show that *Penicillium roqueforti* is well supplied with an enzym capable of hydrolyzing both simple esters and triglycerids.

## CONCLUSIONS

The more important conclusions to be drawn from this investigation are:

(1) During the ripening of Roquefort cheese a considerable amount of the fat is hydrolyzed.

(2) *Penicillium roqueforti* produces a water-soluble lipase, which is the chief factor in the accomplishment of the hydrolysis.

(3) The hydrolysis results in the accumulation of the acids of milk fat in both the free and combined forms.

(4) Of these acids, caproic, caprylic, and capric and their readily hydrolyzable salts have a peppery taste and are responsible for the characteristic burning effect of Roquefort cheese upon the tongue and palate.

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# CORN-LEAF BLOTCH MINER

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## INTRODUCTION

There have heretofore been no references in the literature of economic entomology to *Agromyza parvicornis* Loew (Houser, 1912),[1] the corn-leaf blotch miner; therefore, it might be termed a new enemy of corn (*Zea mays*). While this is literally true, its work has been recorded previously, although credited to another species (Comstock, 1881). Up to the present time it has not proved to be a very serious pest, mainly because of the army of parasites that attack it. It is, however, entirely within the range of possibility that considerable injury may be done in the partial absence of these natural enemies. Every adverse influence tends to decrease the vitality of the plant, and, when a small plant of only three or four leaves has one or two of these destroyed, its metabolizing power is greatly lessened. Even large plants, if subjected to a heavy infestation of this insect, would undoubtedly suffer seriously, as each miner larva is capable of destroying half a square inch of leaf surface; and the injury is permanent, since the tissues die.

## HISTORY OF THE SPECIES

The adult (Pl. III, fig. 1) was described in 1869 by Loew from a male and female from Washington, D. C. The late John B. Smith recorded it from New Jersey in 1909, but without reference to its habits. Prof. C. W. Johnson, of the Boston Museum of Natural History, stated in a letter to Prof. F. M. Webster, of the Bureau of Entomology, that he has collected this species at Niagara Falls, N. Y., and that Mr. S. A. Shaw took it at Hampton, N. H.

In 1879 Mr. Theo. Pergande, of the Bureau of Entomology, reared a dipterous miner from corn leaves collected in Washington, D. C. A record of this rearing was published (Comstock, 1881) under the name *Diastata*, n. sp. This material can not now be located, but the description of the mines would indicate that it was Agromyza and in all probability *A. parvicornis*.

During August, 1883, Mr. Pergande again reared the leaf-miner from corn leaves at Washington, D. C. Each time only a few Diptera were reared, though numerous parasites were obtained. D. W. Coquillett (1898) published the record of this second rearing under the name *Agromyza neptis*. He later wrote a marginal note on his copy of the

[1] Citations to literature in parentheses refer to "Literature cited," p. 30–31.

paper, changing the name to *A. parvicornis;* however, this correction appears never to have been published. The specimens have been examined by Mr. J. R. Malloch, recently of the Bureau of Entomology, who sustains the opinion of Coquillett. This is the only recorded injury that can be fixed with absolute certainty upon this latter species, although there is a record of the rearing of parasites from a leaf-miner on corn at Jacksonville, Fla., and a similar record by Prof. F. M. Webster at La Fayette, Ind.—both in 1886. The miner in both of these instances was probably *A. parvicornis*, as in these two records published in Insect Life[1] the host is given as *Diastata*, n. sp.

Wherever the work of a miner in corn leaves is mentioned the writer will state that it is probably *Agromyza parvicornis*, if the notes clearly state that it is a "blotch miner," since there is no other species of blotch miner known at this time to occur in corn leaves. This will explain why the work is attributed to this species, even when material has not been reared.

Dr. W. D. Hunter, in charge of Investigations of Insects Affecting Southern Field Crops in the Bureau of Entomology, collected this species at Victoria, Tex., in 1903, and Mr. E. S. Tucker, then of the same office, found it at Plano, Tex., in 1907.

The writer's attention was first called to this leaf-miner in June, 1908, at Richmond, Ind. While walking through a cornfield, it was noticed that the tips of some of the leaves appeared colorless, a few having a scorched appearance. Upon closer examination it was found that the tips of these leaves contained footless maggots which were devouring all the tissue between the two surfaces of the leaf, leaving it with a sickly, colorless appearance. Some of these larvæ were reared to adults and determined as *Agromyza parvicornis*.

Mr. C. N. Ainslie, of the Bureau of Entomology, noticed a leaf-miner in corn at East Grand Forks, Minn., in August, 1907, but reared only parasites. This may have been the same species as that found at Richmond, Ind., but, as no description of the mine was given, it is hazardous even to make a guess.

Mr. G. G. Ainslie, of the Bureau of Entomology, observed the work of a miner in corn leaves at Monetta, S. C., in May, 1908, and at Spartanburg and Clemson College, S. C., in June of the same year; but no adult flies were reared, although parasites were reared at Monetta and Clemson College. The species grown at Monetta, at least, was very probably *Agromyza parvicornis*, since its work was described as a "blotch mine." Mr. Ainslie reared adults of this species from corn at Marion, S. C., in May, 1909. Parasites were very numerous here, and only a few miners completed their development. A number of parasites were reared by Mr. Ainslie from a leaf-miner in corn leaves at Hurricane Mills, Tenn.,

[1] Some of the bred parasitic Hymenoptera in the national collection. U. S. Dept. Agr., Div. Ent., Insect Life, v. 2, no. 11/12, p. 348–353. 1890. "*Bracon diastatae* Ashm.," p. 348.

in 1911. From the appearance of the mines and pupa the host was probably *A. parvicornis*. He also reared adults of this miner from corn at Montgomery, Ala., in 1911 and at Lakeland, Fla., in 1912.

Mr. T. H. Parks, recently of the Bureau of Entomology, observed the work of a leaf-miner on sweet corn at Wellington, Kans., in 1909. While nothing but parasites was reared, the character of the mine indicates that it was probably the work of *Agromyza parvicornis*.

This species was again found at Richmond, Ind., but sparingly, in 1909, 1910, and 1911. In October, 1911, it was found infesting broom or hog millet (*Panicum miliaceum*) at La Fayette, Ind., and in 1912 it was very abundant in corn and several of the grasses on the experiment station grounds at La Fayette. In fact, it could be readily found in any cornfield in that locality.

Mr. J. J. Davis, of the Bureau of Entomology, found it at Lancaster, Wis., mining in corn leaves, and at Danville, Ill., in the leaves of *Echinochloa crus-galli*, in July, 1912. Mr. C. N. Ainslie reared the same miner from corn at Salt Lake City, Utah, in 1912; and Mr. W. L. McAtee, of the Biological Survey, collected it at Biltmore, N. C., the same year. It appears to have been more abundant in 1912 than during any of the other years, and naturally more species of parasites were reared at that time than from all previous rearings. Mr. Davis also found the work of what is probably this species near Louisville, Ky., in July, 1913.



FIG. 1.—Map of the United States, showing distribution of *Agromyza parvicornis*, the corn-leaf blotch miner. (Original.)

## DISTRIBUTION OF THE CORN-LEAF BLOTCH MINER

From the foregoing it will be seen that this leaf-miner has quite a wide range, being found as far north as Wisconsin, as far east as Washington, D. C., and New England, as far south as Alabama and Florida, and as far west as Salt Lake City, Utah, as well as in Texas. It probably occurs throughout the United States wherever corn is grown. The known distribution is shown on the map (fig. 1).

## HOST PLANTS

There does not seem to be a great variety of host plants, *Agromyza parvicornis* having apparently thus far confined itself to a few species of the Gramineæ. The species seems to show a preference for corn, especially

the young plants, although it is very partial also to some of the broad-leaved millets. On corn it breeds continuously throughout the season. The writer found larvæ in mines in corn leaves from May until severe frosts killed the plants in the fall. Next to young corn the blotch miner apparently prefers the broad, hairy-leaved varieties of millet, such as *Panicum miliaceum*, although it has been reared from several varieties of the smooth-leaved millets. Occasionally it will be found in crab-grass (*Panicum sanguinale*), and it breeds readily in barnyard grass (*Echinochloa crus-galli*). It would not be at all surprising if in later years it should be reared from wheat and oats, as apparently the same kind of larval mine has been found in these plants, although the adults have not been obtained.[1]

## CHARACTER OF INJURY

In young corn plants and in the small grasses and grains the larvæ work from the tip of the leaf toward the base, devouring all of the tissue between the upper and lower epidermis (Pl. I, figs. 2, 3). The mandibles of the larva consist of two chitinized hooks, which are used in much the same manner as a hoe, portions of the thin layer of tissue being scraped from between the two surfaces of the leaf with every stroke. In small plants the larvæ work the entire width of the leaf, leaving only the epidermis of the upper and lower surfaces. Plate I, figure 2, shows a young corn leaf taken from a cage, with six larvæ at work inside. Soon after the work is done the leaves have a colorless appearance, and in a few days they turn brown and curl up. One larva is sufficient to destroy a young corn leaf, although as many as four have been found in a single leaf in the field, and as many as eight or ten have been found in a leaf in the rearing cages. Often there is not sufficient nourishment in a single leaf for the development of all when they are reared in confinement, in which case some perish. Sometimes in the field two or more leaves of a young corn plant are attacked when the plant is only a few inches high and has, therefore, few leaves. It can be readily seen that corn might suffer severely in a cold, backward season with this pest abundant.

Instances have been noted where the tip of the mined leaf has been almost completely filled with water, although the larva may still be inside and apparently not seriously inconvenienced. Plate I, figure 3, shows such an instance, the epidermis of the upper and lower surfaces being separated for a distance of about one-fourth of an inch. The mines become filled in this way only when the weather is very rainy. In cases of this kind the larvæ do not feed much until the water has evaporated, although under normal conditions they appear to feed continuously, the writer having observed them both night and day and never having seen them at rest for more than a few seconds at a time.

[1] It might be well to state in this connection that Asa Fitch (1856) reared and described a new species of Agromyza (*A. tritici*) from New York, calling it "the wheat mow fly." After reading the description in Fitch's article and comparing it with the work of the miner at La Fayette, Ind., one readily sees that two species are involved.

The leaves of the grasses and smaller grains are affected in the same manner as the leaves of young corn, but as they are much smaller they can not accommodate so many larvæ. This miner is not able to go down the base of one leaf and up into another as is *Ceradontha dorsalis* Loew; therefore it has to depend entirely upon the nourishment to be derived from one leaf. *C. dorsalis* makes a long, narrow mine, working toward the base of the leaf, and in small plants it may go down the leaf sheath and work out into another leaf.

From the time corn leaves are an inch or more in diameter until they are mature the larvæ have plenty of room for development. The mines take on a different character in large leaves. In these they may start at any point along the leaf. Sometimes several larvæ will hatch in close proximity, and the mines will coalesce, forming a large blotch, which may be several inches in length and nearly an inch across. A mine like this is shown in Plate I, figures 5 and 6. At a distance of 10 or 15 yards such mines show up very distinctly against the dark-green background as large grayish or whitish blotches. Where there is only one larva to a mine in these large leaves the mine may be a comparatively long, narrow one (3 to 4 inches in length), gradually enlarging, the last third to half forming a blotch. In other instances the mine may be 3 inches in length and about three-eighths of an inch across for nearly the entire length, ending in a blotch about one-half inch in diameter, as shown in Plate I, figure 7. The mines may, however, form blotches, as shown in Plate I, figure 1.

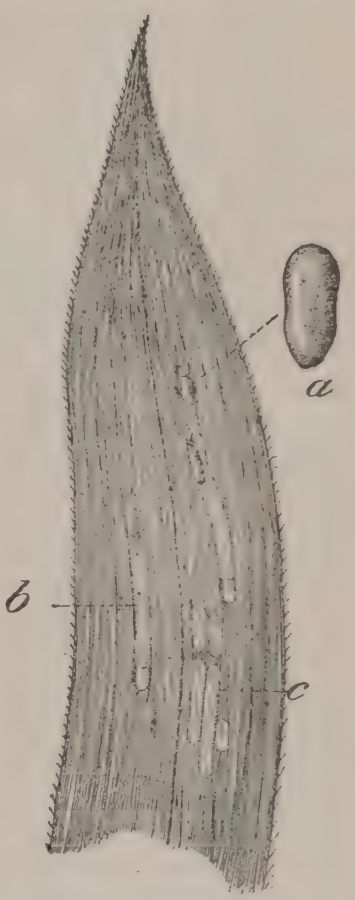



FIG. 2.—Tip of corn leaf, showing work of *Agromyza parvicornis*, the corn-leaf blotch miner: *a*, Egg, greatly enlarged; *b*, newly hatched larva in mine; *c*, feeding punctures of adults. Much enlarged. (Original.)

When the plants become older and tougher, the miners do not devour all of the tissue between the two leaf surfaces. In this case a miner that is working in the underside of the leaf would scarcely be noticed from the upper surface. Therefore, the greatest injury is wrought when the plants are young. If very abundant, however, the larvæ could cause serious injury to corn in advanced stages of growth, provided their parasitic enemies were not present in sufficient numbers to hold them in check.

## DESCRIPTION OF AGROMYZA PARVICORNIS

### THE EGG (FIG. 2, *a*)

The egg is milky white and flattened from above and below. It is from 0.4521 to 0.5043 mm. in length and from 0.1739 to 0.1913 mm. in width. It is broadly rounded at each extremity and slightly constricted at the center. The anterior extremity is slightly more pointed and somewhat more flattened. The surface of the chorion is smooth and apparently without any markings whatever.

Mr. J. R. Malloch, formerly of the Bureau of Entomology, has kindly consented to draw up descriptions of the larva and pupa. He has redescribed the adults also. His descriptions follow.

## THE LARVA (FIGS. 3 AND 4)

In color the larva is pale greenish white, becoming, as it nears maturity, more yellowish white. The average length when mature is about 3 mm. and the breadth about 1 mm. The segments are but poorly defined and under a strong magnification show on the surfaces shallow, closely placed punctures, in each of which is situated a very minute hair. The mouth parts are heavily chitinized, deep black, and, while capable of being entirely retracted, are always visible, as they show clearly through the semitransparent larval skin. The spiracles of the anterior pair are small and slightly darker than the general color of the larva; the posterior pair, which are more or less embedded in the body, are closely approximated and placed at the extreme end of the body. On the ventral surface close to the anal extremity there is a vestigial sucker-like foot.




FIG. 3.—*Agromyza parvicornis*, the corn-leaf blotch miner: Full-grown larva; *a. sp.*, anterior spiracles; *p. sp.*, posterior spiracles. Much enlarged. (Original.)

## THE PUPARIUM (FIG. 5)

In color the puparium is reddish brown. The length averages about 3 mm. and the breadth slightly over 1 mm. The segments are well differentiated, without any ornamentation or punctation. The spiracles of the anterior pair are prominent and protruding, those of the posterior pair (fig. 5, *c*) much more closely placed and less distinct. In shape the puparium is as represented in figure 5, *a* and *b*.

## THE ADULT (PL. III, FIG. 1)

*Agromyza parvicornis* Loew, 1869, *in* Berlin. Ent. Ztschr., Jahrg. 13, No. 92, p. 49.

MALE AND FEMALE.—Frons black or black brown, opaque; orbits slightly shining black, four orbital bristles present; orbits differentiated from center stripe, bristles situated nearer inner than outer margin of orbits, a few weak hairs in an irregular row laterally beyond them. Antennæ brown or brownish black, rather below the normal size; third joint short, rounded in front, thickly covered with short, soft, whitish pilosity; arista brown, generally yellowish near base, except on the short thickened portion, which is glossy black; pubescence very close, generally distinct; length of arista equal to the distance from its base to the upper orbital bristle. Face brown, nearly perpendicular in profile, the central keel slight; cheeks brown or yellowish brown, very much higher posteriorly than anteriorly, at highest part one-third as high as eye; marginal bristles numerous; vibrissæ differentiated but not very strong; proboscis brown; palpi black, very slightly dilated, weakly bristled. Mesonotum glossy black; disk thickly covered with short setulæ; two pairs of dorso-centrals present; the bristles between the posterior pair distinct; pleuræ, scutellum, and postnotum concolorous with disk of mesonotum, pleural sutures rarely, and beneath wing bases generally yellowish; squamæ whitish yellow, fringes brown. Abdomen colored as the thorax; ovipositor of female as in Plate III, figure 1, *E;* hypopygium of male as in Plate III, figure 1, *D*. Legs black, the tibiæ and tarsi sometimes paler, brownish



FIG. 4.—*Agromyza parvicornis*, the corn-leaf blotch miner: Mouth hooks of larva. Greatly enlarged. (Original.)

yellow, most distinct on knee joints; mid tibia with the posterior bristles distinct. Wings clear, slightly grayish on anterior half; venation as in Plate III, figure 1, *A;* halteres yellow, the knob whitish. Length, 3 to 4 mm.

Originally described from the District of Columbia (Osten-Sacken).

## LIFE HISTORY OF AGROMYZA PARVICORNIS

### OVIPOSITION

The act of oviposition has never been observed by the author, although the females have often been seen making feeding punctures which are apparently the same as egg punctures.

The eggs, which have been observed in the leaves repeatedly, may be deposited either from the upper or lower surface. The females always choose the tip of the leaf in small plants when ovipositing in the field, but in large plants the eggs may be placed at any point on the leaf. In confinement they seek the tips of the small leaves also, but as there is necessarily a small amount of leaf surface in a small cage, there are often as many as 15 eggs deposited in one leaf at different points. The eggs are inserted with the long axis parallel to the veins of the leaf. Figure 2, *a*, represents the eggs in situ and also greatly enlarged.




FIG. 5.—*Agromyza parvicornis*, the corn-leaf blotch miner: Puparium; *a*, dorsal view; *b*, ventral view; *c*, view of posterior extremity. Much enlarged. (Original.)

In making the puncture the fly forces the point of her abdomen downward, rearing the anterior portion of her body slightly, and touches the tip of the abdomen to the leaf, whereupon the small lancets, which apparently make up the ovipositor, are put in motion. The lancets appear to slide past each other, with their cutting edge in the plant tissues, thus acting somewhat like a saw. They are forced down between the two surfaces of the leaf and a strip of the epidermis about 0.3 mm. in width and about 0.9 mm. long is pushed back; the egg is probably inserted then, and this flap is in some way brought back over the egg and fastened down, probably with a mucilaginous substance. Sometimes it appears as though both ends of the egg were covered, although eggs are often seen to be only partly covered. These eggs can be readily detected with the naked eye when only a few feet away, as they show as milky white spots against the green background.

When only feeding, the female, after making the puncture, steps backward a few paces, inserts her proboscis into the puncture, and sucks the juices. The male has never been observed feeding at these punctures. The feeding punctures are very numerous, sometimes small leaves being

riddled even in the fields. Plate I, figure 4, illustrates this work in a leaf of millet as it occurs in the fields. In fact, in almost any place in the fields where eggs may be found there are usually one or more of these feeding punctures near by. Figure 2, *a*, *b*, *c*, represents the feeding punctures, eggs, and newly hatched larvæ as one often finds them in the field. These spots or punctures finally dry out and turn almost white.

Oviposition is, in all probability, accomplished during the day. Observations were made several times at night, but the adults were almost always at rest upon the plant at the top of the cage.

### PERIOD OF INCUBATION OF THE EGG

No exact incubation period can be given, since this depends mainly upon the temperature. It is quite short in the summer, being about 80 hours in the latter part of July. In the latter part of September and the first part of October the eggs were not less than five days in hatching, and perhaps slightly longer. This would be nearly two days longer than the time required for hatching in July.

### LENGTH OF LARVAL STAGE

The larval period, like the egg stage, varies considerably in length. In the middle of the summer larvæ will attain full growth in four days, while in the cooler weather of spring or fall they will require 10 days or more. Some larvæ hatched the first week in October and were overtaken by frosts.

### LARVAL HABITS

When ready to hatch, the larva ruptures the eggshell at the cephalic extremity and begins to feed, gradually working its way out of the shell into its excavated burrow. It can not leave one leaf and enter another, and if removed from the leaf it will die. If a full-grown larva be taken from its burrow and placed upon a hard surface, it will bring each extremity beneath its body, then suddenly straighten to its full length, and thus skip about over the surface like a cheese maggot Larvæ will continue this skipping for a considerable time, often jumping an inch high, apparently hunting for a hiding place. They may remain entirely submerged in water for a day or more and still recover and pupate normally. Excrement is voided at intervals within the mine, but at no time has a cast skin been found or other evidence of molting. The larva apparently punctures one of the walls of the mine and drops to the ground to pupate, going down sometimes 2 inches if the soil be sandy; in ordinary soil it will go only from one-fourth to one-half inch below ground. Larvæ will pupate on a solid surface in the open if they can not reach the ground or find cover. When ready to pupate the larva contracts in length, the segments becoming quite distinct. It then turns a cream color, gradually darkening until it becomes a reddish brown.

### LENGTH OF PUPAL STAGE

The pupal stage varies from 14 days in warm weather to 22 days in spring and fall. In the late summer of 1912 there were some puparia that did not disclose adults, and upon examination they were found to be in good condition in November. These puparia were kept indoors and disclosed adults in January and February. They were of the generation that pupated from the middle to the latter part of August. This would seem to indicate that some go into hibernation rather early in the fall.

### LIFE AND HABITS OF THE ADULTS

The flies begin to issue during the latter part of May. The males will not live more than three or four days in confinement, whereas the females will easily live two weeks when the weather is not too hot. It will thus be readily seen why the generations overlap so completely, since the progeny of one individual may be in the pupal stage before she ceases oviposition. The males apparently do not feed, though the females do considerable feeding, as evidenced by the punctures in the leaves shown in Plate I, figure 4. Both males and females, however, appear to be fond of water and seem particularly thirsty soon after issuing. In midsummer the adults appear to be most active in early forenoon and late afternoon. In midday they appear to seek shelter on the underside of leaves.

### NUMBER OF EGGS DEPOSITED BY ONE INDIVIDUAL

The female flies begin oviposition in 5 to 10 days after emergence, if males be present; otherwise they will refuse to oviposit. There are no very complete observations on egg laying. Several individuals were observed, but for various reasons the records are not complete for their whole life. They have been found to deposit from 30 to 60 eggs, but will doubtless deposit more than this throughout the whole period. Dissections give no satisfactory clue to the number of eggs that may be deposited. The species will apparently breed as freely in confinement as out of doors.

### NUMBER OF GENERATIONS

It is almost impossible to get a clue to the number of generations by making observations in the field, since they overlap so completely. After the first generation, if the insects are at all abundant, eggs, larvæ, and adults can be found in the field simultaneously throughout the remainder of the year. Therefore, early in the spring of 1912 a series of rearings was begun in confinement, and this series was continued until freezes killed off the vegetation in the fall. It was found that there were four complete generations and a partial fifth. It is possible that the insects may breed more rapidly in the open fields and that if they could be readily followed it would be found that there are five generations.

### HIBERNATION

The first freezes in autumn killed the adults in the rearing cages at La Fayette, Ind., in 1912. Any larvæ that happened still to be feeding were killed also. This indicates very strongly that the species does not hibernate in either the adult or larval stage. It appears, then, that in the latitude of La Fayette, Ind., at least, it passes the winter in the puparium only.

### LIFE HISTORY OF AGROMYZA PARVICORNIS IN FLORIDA

Mr. G. G. Ainslie made observations on this miner from November, 1912, to the middle of April, 1913, at Lakeland and Orlando, Fla. Special plantings of corn were made in the field, and volunteer plants and regular plantings were also kept under observation.

### SEASONAL OCCURRENCE OF LARVÆ

On November 26, 1912, larvæ were found to be quite plentiful in volunteer corn that was about 10 to 12 inches high. This corn was kept under observation, and it was found that the miners bred slowly here throughout the winter. No killing frosts occurred during this time. There were three dates during this period at which the larvæ were the most abundant—viz, February 4, March 5, and March 27.

### PERIOD OF INCUBATION OF EGGS

Of 19 eggs that were deposited in confinement during March, 2 hatched in 6 days and 17 in 7, while some that were found in the field did not hatch for 8 days, the average being 6.5 days.

### LENGTH OF LARVAL STAGE

Accurate observations were made on 13 larvæ during the latter part of February and to the middle of March. It was found that the period from hatching until they left the mine varied from 6 to 12 days, the average for the 13 being 7.9 days. The larvæ in Florida, as well as farther north, invariably leave the mines to pupate.

### LENGTH OF PUPAL STAGE

Table I gives most of the available data on the length of the pupal stage for Florida.

It will be noted that the larval stage was longer in Florida than given for Indiana. Larvæ that hatched late in Indiana were overtaken by frosts, while the rearings in Florida continued through cool weather without being interfered with by frosts.

The same interesting phenomenon occurred here that was observed in Indiana—namely, that apparently healthy living pupæ went into hibernation at a time when the miner was found breeding freely in the same

locality. Some individuals that pupated in January and February were apparently alive and in good condition as late as the 25th of April.

TABLE I.—*Length of pupal stage of Agromyza parvicornis in Florida.*

| Number of individuals. | [1] Pupated— | Adult emerged— | Length of stage. |
|---|---|---|---|
| | | | *Days.* |
| 1 | Feb. 1 | Mar. 4 | 32 |
| 1 | 1 | 4 | 32 |
| 1 | 20 | 16 | 24 |
| 1 | 20 | 19 | 27 |
| 1 | Mar. 8 | 30 | 22 |
| 1 | 13 | Apr. 3 | 20 |
| 6 | ..... | ..... | 157 |
| Average length of pupal stage for 6 individuals, 26.2 days. | | | |

[1] Two individuals pupated on Jan. 24 were still alive and healthy on Mar. 22.

In Indiana, as stated previously in this paper, individuals that pupated in the middle of August, 1912, and had been kept indoors since fall, disclosed adults in January and February, 1913.

The Florida data show conclusively that this insect will breed continuously, except in a few isolated instances, throughout the year, if its host plants are supplied and no freezing temperatures are encountered.

## REARING METHODS

Several kinds of rearing cages were tried in this work, but it was found that the use of the one shown in Plate II, figure 2, was attended with the greatest success. It consists of a 12-inch flowerpot and a collar made of fine-mesh brass strainer wire with supports of galvanized iron, into which is fitted a large electric-light globe—the kind used on street lights—the top of which is covered with cheesecloth. There is a free circulation of air through the cage and moisture does not collect on the sides so readily as in other types, thus giving more nearly normal conditions. These cages were kept under a shelter so that direct rays of the sun did not strike them, and the miners were very easily reared in them. Plate II, figure 1, illustrates the rearing shelter with the cages in place.

## PARASITIC ENEMIES

There are 18 species of hymenopterous parasites that may be said with reasonable certainty to attack *Agromyza parvicornis*, since there is as yet no proof that any are secondary parasites. Three of these are

braconids and 15 chalcidoids. Nine of these chalcidoids are known to be new species, and they have recently been described by Mr. J. C. Crawford, Associate Curator of the Division of Insects, United States National Museum. Three others are probably new, but as there is only a limited amount of material they can not be recorded as new with certainty. Two of the braconids are new also.

With this army of parasites in the field it will be readily seen that the chances that the miner will do serious mischief are reduced to a minimum. Only under exceptional circumstances would *Agromyza parvicornis* be able to elude this array of enemies. This is a very good illustration of the holding in check by its natural enemies of what would otherwise in all probability be a pernicious insect, thus showing that it is entirely possible for a group of parasites under favorable conditions to control their host insect.

The life history of none of these parasites has been worked out completely.

Of these parasites *Derostenus diastatae* is by far the most abundant and probably the most important. *Diaulinus websteri* and *D. begini* have also been reared quite plentifully and are probably next in importance. An effort is here made to discuss these parasites in the order of their importance without regard to their systematic relationship.

**Derostenus diastatae** How.—This chalcidoid very closely resembles *D. punctiventris* (Pl. V, fig. 1). It is an internal parasite and was first reared in 1879 from a corn-leaf miner which was called at the time *Diastata*, n. sp., but which has since been determined as *Agromyza parvicornis*. The parasite was described by Dr. L. O. Howard as *Entedon diastatae*. It appears to have been very abundant at that time. Prof. Comstock (1881) writes:

> During the season of 1880 these leaf-miners were extremely difficult to find, which was doubtless owing to the very extensive parasitization of the 1879 individuals. Out of thirty or forty specimens examined but one contained a sound larva, which was reared to maturity. All the rest contained several minute parasitic larvæ.

Prof. F. M. Webster reared this parasite in 1886 at La Fayette, Ind., from what was probably this miner. He reared large numbers of the same species at Urbana, Ill., in 1902, from a miner in grass. It was reared by Mr. C. N. Ainslie at Washington, D. C., in 1907, and by Dr. H. Kraemer, of Philadelphia, in 1905. In these two last instances it was parasitic upon a miner in grass leaves. Mr. G. G. Ainslie reared what is doubtfully the same species at Clemson College, S. C., in 1908. The host in this instance was probably *Agromyza parvicornis*, as it was a blotch miner in corn. He also reared it in abundance in 1911 and 1912 from a miner in corn leaves at Hurricane Mills, Tenn. Mr. T. H. Parks reared numbers of this parasite from a blotch miner in corn leaves at Wellington, Kans., in 1909. The same parasite was reared in abundance by the author from *A. parvicornis* at Richmond, Ind., in 1911, and at

La Fayette, Ind., both by the author and by Mr. Philip Luginbill, in 1912, while it was reared by Mr. G. G. Ainslie at Lakeland, Fla., and by Mr. J. J. Davis at Danville, Ill., in the same year. This parasite is thus known to cover a pretty wide range, and future rearings may show it to be present wherever its host is found. While nothing definite is known of its life history, it appears that the complete life cycle approximates that of its host. The larvæ of the parasite kill the larva of the host at or about the time the latter reaches maturity. Sometimes as many as eight of the larvæ of the parasite are found in one host larva. As soon as these are grown, they leave the body of the host and crawl out into the gallery a short distance to pupate. When they first pupate they are white, but later they turn black.

**Diaulinus pulchripes** Cwfd.—Mr. J. C. Crawford (1912) has recently described this species from two specimens in the Ashmead collection from Algonquin, Ill. Prof. Webster reared three specimens of this species from a miner in grass at Urbana, Ill., in August, 1902. The author reared a number of specimens from *Agromyza parvicornis* at La Fayette, Ind., in 1912. Nothing is known of its life history.

**Diaulinus websteri** Cwfd.—This species was reared quite plentifully from *Agromyza parvicornis* in corn leaves at Salt Lake City, Utah, in 1912, by Mr. C. N. Ainslie. It was recently described by Mr. Crawford (1912), the habitat being given as Tempe, Ariz.

**Zagrammosoma multilineata** Ashm.—Ashmead (1888) described this parasite under the genus Hippocephalus in 1888. The specimens were reared in 1887 from *Lithocolletis ornatella* Chambers on locust. Prof. Webster reared it from a species of Lithocolletis from Ohio in 1893. The author reared it from *Agromyza parvicornis* in 1912 at La Fayette, Ind. Nothing is known of its life history. Repeated attempts were made by the author to rear it, as it appeared to be quite common, but unless the pupæ were collected just before the adults were ready to issue they would not emerge.

**Sympiesis** sp.—This parasite was quite common at La Fayette, Ind. It seems, however, that for some unknown cause only a very few females were reared; consequently they have not been determined specifically. Doubtless they are new. Prof. Webster reared *Sympiesis nigripes* Ashm. from a lepidopterous leaf-miner in bur oak in Ohio in 1893. Mr. Parks reared a species of Sympiesis in 1909 from a blotch miner in corn at Wellington, Kans. Mr. G. G. Ainslie also reared it from the corn-leaf blotch miner at Hurricane Mills, Tenn., in 1912. The author reared it from *Agromyza parvicornis* at La Fayette, Ind. It is an internal parasite, but further than that nothing is known of its life history.

**Closterocerus tricinctus** Ashm.—This species (Pl. IV, fig. 1) was described by Ashmead (1888) under the genus Pleurotropis, with the statement that it was reared from a Lithocolletis larva on sycamore. Prof. Webster reared it from a miner in *Panicum multifolia* at Urbana,

Ill., in 1902, and Mr. G. G. Ainslie reared it from a miner in corn leaves at Hurricane Mills, Tenn., in 1911. The host in both instances may have been *Agromyza parvicornis*. The author reared it sparingly from *A. parvicornis* at La Fayette, Ind., in 1912.

**Closterocerus utahensis** Cwfd.—Mr. C. N. Ainslie reared this species from *Agromyza parvicornis* at Salt Lake City, Utah, in 1912. This is the only record of its attacking this miner. It had been reared from *A. pusilla* Meig. previous to this. Mr. Crawford (1912) described this species, giving the type locality as Salt Lake City, Utah.

**Derostenus punctiventris** Cwfd.—This is an internal parasite. It was reared by the author sparingly from *Agromyza parvicornis* at La Fayette, Ind., in 1912. Previously it had been reared from *A. pusilla* from several localities by other members of the force. It is a new species, and Mr. Crawford (1912) has recently described it, giving Salt Lake City, Utah, as the type locality. Plate V, figure 1, represents the adult of this species, which closely resembles *Derostenus diastatae*.

**Diaulinus begini** Ashm.—Mr. C. N. Ainslie reared this species sparingly from *Agromyza parvicornis* in corn leaves at Salt Lake City, Utah, in 1912. The author reared it from *A. pusilla* at La Fayette, Ind., in 1911. This species was described by Ashmead, and there was only one specimen in the National Museum collection previous to the recent rearings from Agromyza.

**Notanisomorpha ainsliei** Cwfd.—This parasite was reared sparingly from *Agromyza parvicornis* at Salt Lake City, Utah, in 1912, by Mr. C. N. Ainslie. It is a new species and was recently described by Mr. Crawford (1912). It has other hosts than *A. parvicornis*.

**Cirrospilus flavoviridis** Cwfd.—This is a new species and has recently been described by Mr. J. C. Crawford (1913). It has been reared from *Agromyza parvicornis* and *A. pusilla* at Salt Lake City, Utah, by Mr. C. N. Ainslie. Nothing is known of the life history.

**Chrysocharis ainsliei** Cwfd.—A few specimens of this parasite were reared from *Agromyza parvicornis* in corn leaves by Mr. C. N. Ainslie at Salt Lake City, Utah, in 1912. It has been reared from other species of Agromyza. It was recently described by Mr. Crawford (1912, p. 174), the type locality being given as Salt Lake City, Utah.

**Chrysocharis parksi** Cwfd.—One specimen of this parasite was reared from *Agromyza parvicornis* in corn leaves by Mr. C. N. Ainslie at Salt Lake City, Utah, in 1912. It was described in 1912 by Mr. Crawford (1912).

**Pleurotropis utahensis** Cwfd.—Mr. Crawford's (1913) description of this new species has recently been published. One specimen was reared by Mr. C. N. Ainslie from *A. parvicornis* in corn leaves at Salt Lake City, Utah. Mr. Ainslie also reared it from a species of Cephus in *Elymus* sp. in the same locality. Text figure 6 and Plate III, figure 2, represent the larva and adult, respectively.

**Pteromalidæ.**—There are at least two species of parasites belonging to the Pteromalidæ that have been reared from *Agromyza parvicornis* at Richmond and La Fayette, Ind. Both species may be and probably are new, but as there is only a limited amount of material they can not be placed with certainty. These parasites are mounted under Webster Nos. 3814 and 3857.

**Opius diastatae** Ashm.—This parasite (Pl. IV, fig. 2) was reared from a corn leaf-miner at La Fayette, Ind., in 1886, by Prof. Webster, and from a miner in corn leaves at Jacksonville, Fla., the same year by Ashmead. It was described by Ashmead (1888) as *Bracon diastatae*, and Gahan (1913) has placed it under the genus Opius. It is recorded in Insect Life[1] as being reared from *Diastata*, n. sp., which was very probably *Agromyza parvicornis*.

**Opius succineus** Gahan.—This is a new species recently described by Mr. A. B. Gahan (1913) of the Bureau of Entomology (Pl. IV, fig. 3). It was recorded from puparia by the author and Mr. Philip Luginbill from La Fayette, Ind., in 1912, and by Mr. J. J. Davis from Danville, Ill., during the same year. It was not very abundant, and nothing is known of its life history.

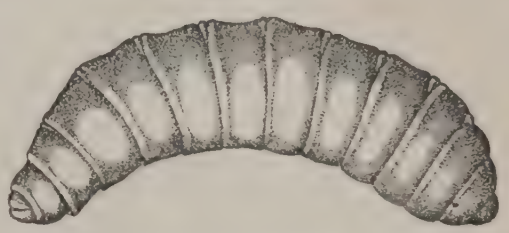


FIG. 6.—*Pleurotropis utahensis*, a parasite of *Agromyza parvicornis:* Larva. Much enlarged. (Original.)

**Opius utahensis** Gahan.—This species (Pl. V, fig. 2) is new also, having been described recently by Mr. Gahan (1913) from Salt Lake City. Mr. C. N. Ainslie reared this species sparingly from the locality just cited, from *Agromyza parvicornis*. Nothing is known of its life history.

**Elachertinæ.**—An unidentified species of the subfamily Elachertinæ was reared from a miner in *Panicum multifolia* at Urbana, Ill., in 1902, by Prof. Webster. As large numbers of *Derostenus diastatae* were reared from the same host at the same time and as this latter is the most important parasite of *Agromyza parvicornis*, it was thought well to mention in this connection the rearing of this unidentified species, which bears Webster No. 1896.

**Macroglenes** sp.—Mr. C. N. Ainslie reared a species of Macroglenes from a leaf-miner in corn at East Grand Forks, Minn. There is no way of knowing what miner was concerned, and this species as well as the foregoing is listed here mainly to establish a record of the rearings. The specimens bear Webster No. 4309.

## REMEDIAL MEASURES

As no occasion demanding remedial measures has thus far arisen, little can be said on this subject. The ordinary means of control would appear

[1] Some of the bred parasitic Hymenoptera in the national collection. U. S. Dept. Agr., Div. Ent., Insect Life, v. 2, no. 11/12, p. 348–353, 1890. "*Bracon diastatae* Ashm.," p. 348.

to offer very little assistance, and it is difficult under such circumstances to suggest measures that would be effective when there has been no opportunity to put any of them into practice or to test their efficiency.

With such a host of parasites as are listed in the preceding pages constantly on the watch we need not concern ourselves seriously about remedies so long as conditions continue as they now are. In the event that a combination of circumstances should occur that would restrain the parasites and give free rein to their host, the blotch miner would undoubtedly prove a pest very difficult of control.

This species seems to furnish an instance in which only the barrier of parasites stands between the farmer and what may easily become, temporarily at least, a very serious pest.

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## PLATE I

Fig. 1.—Large leaf showing blotch mines. Note feeding punctures and two eggs that have recently hatched, with short mines leading out from them.

Fig. 2.—Young corn leaf from breeding cage containing six larvæ.

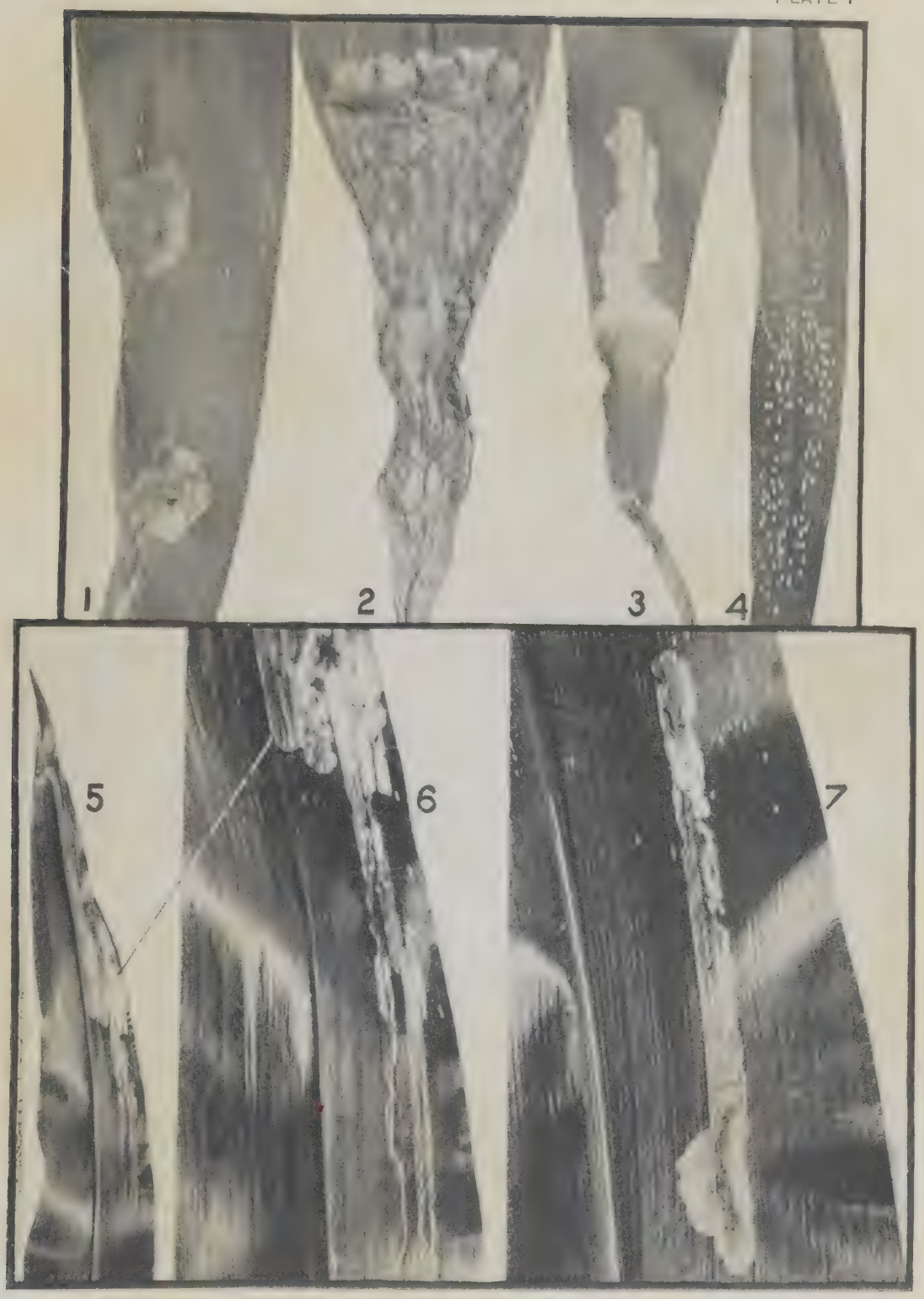
Fig. 3.—Shows how mines occasionally fill with water.

Fig. 4.—Millet leaf showing feeding punctures. All about natural size.

Fig. 5.—Large corn leaf with three larvæ in the same tunnel; reduced. Note point of origin of mines.

Fig. 6.—Section of leaf shown in figure 5 slightly enlarged.

Fig. 7.—Straight mine ending in a slight blotch in a large leaf; slightly enlarged.


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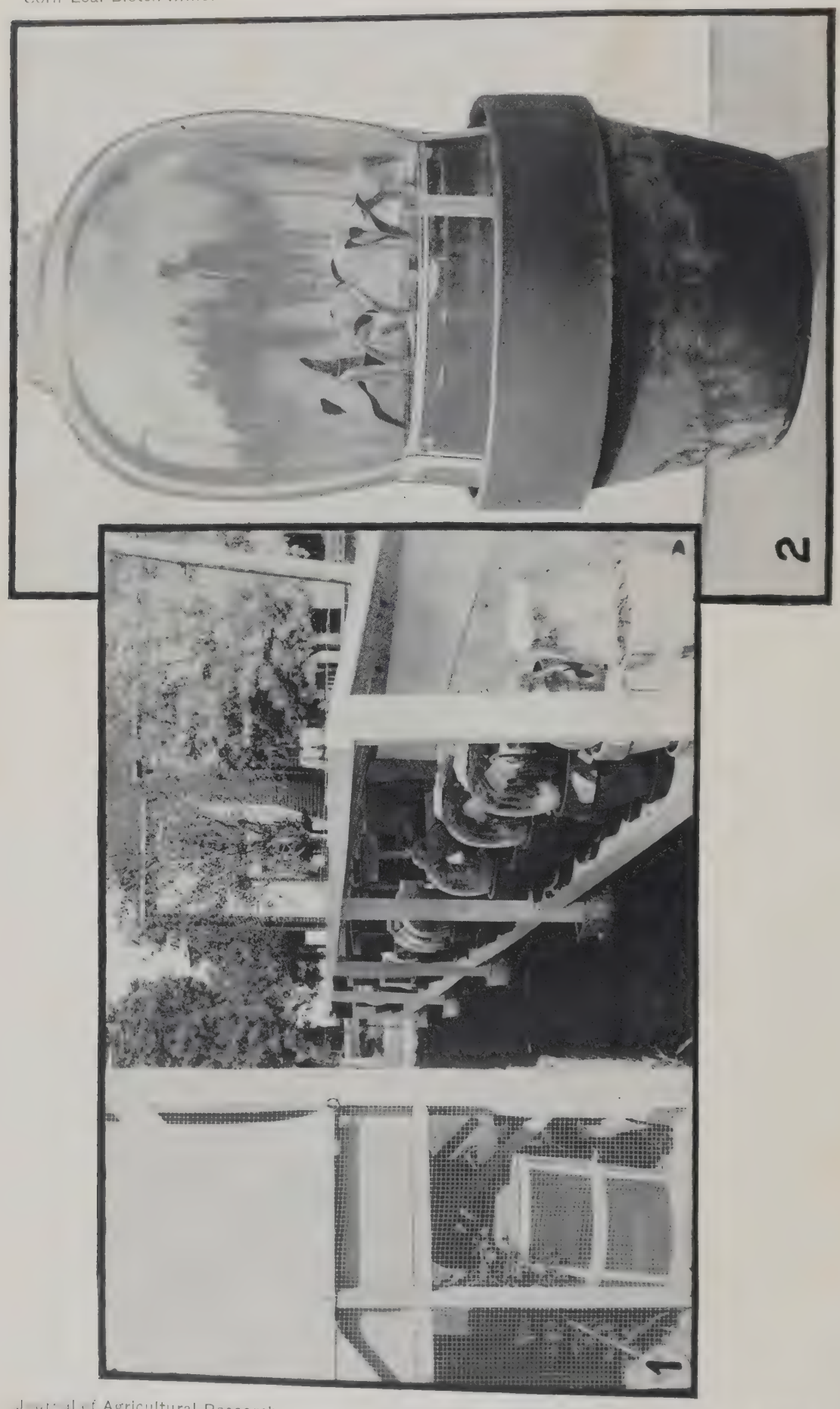

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PLATE II

Fig. 1.—Rearing shelter used for *Agromyza parvicornis* and other insects at La Fayette, Ind.

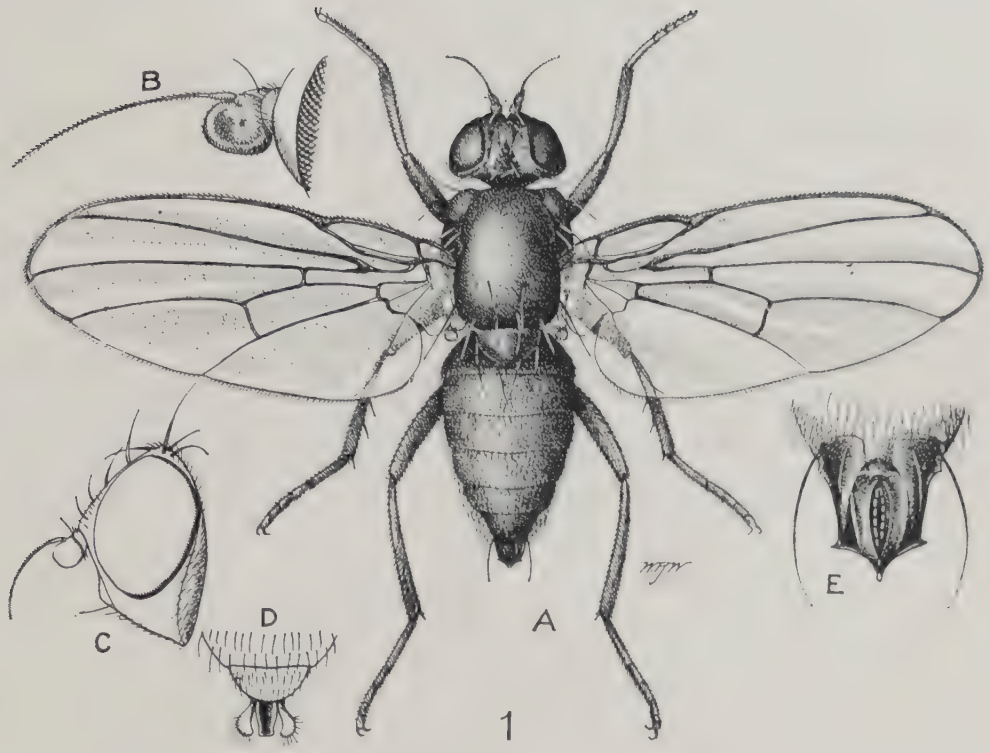
Fig. 2.—Rearing cage used for *Agromyza parvicornis*.

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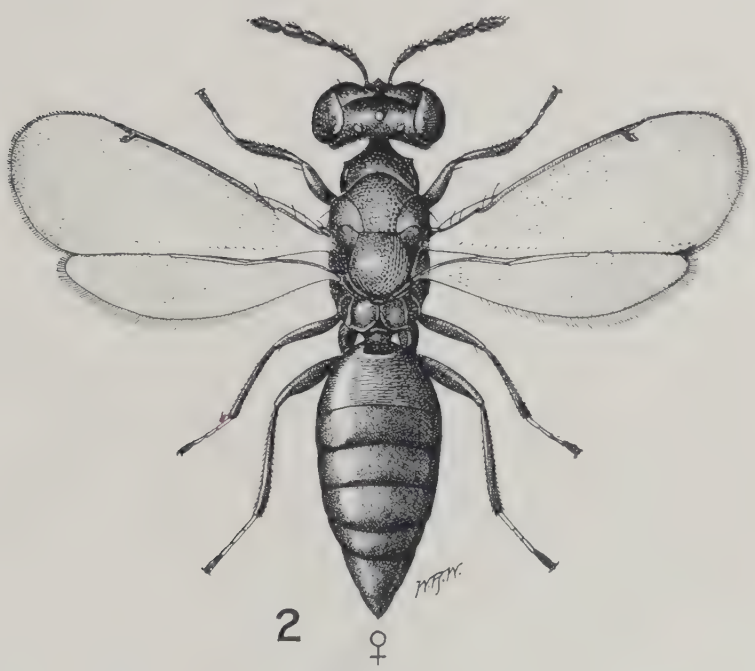
## PLATE III

Fig. 1.—*Agromyza parvicornis*, the corn-leaf blotch miner: *A*, Dorsal view of adult; *B*, antenna of female; *C*, head of male; *D*, hypopygium of male; *E*, ovipositor of female. Much enlarged. (Original.)

Fig. 2.—*Pleurotropis utahensis*, a parasite of *Agromyza parvicornis:* Adult. Much enlarged. (Original.)



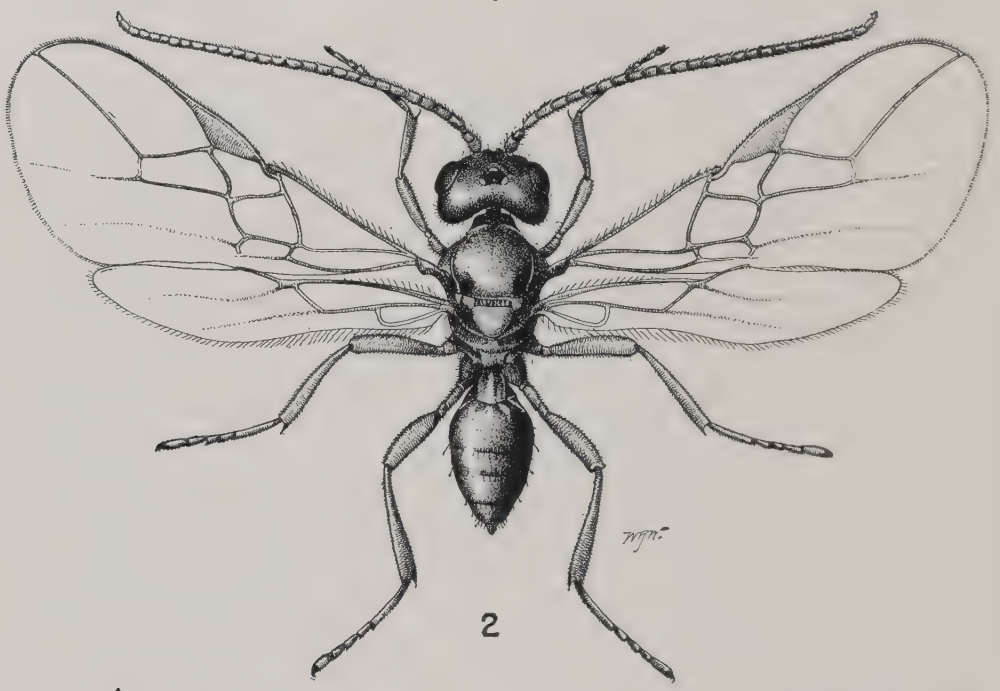

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## PLATE IV

Fig. 1.—*Closterocerus tricinctus*, a parasite of *Agromyza parvicornis:* *A*, Dorsal view of adult; *B*, side view of antenna. Much enlarged. (Original.)

Fig. 2.—*Opius diastatae*, a parasite of *Agromyza parvicornis:* Adult. Much enlarged. (Original.)

Fig. 3.—*Opius succineus*, a parasite of *Agromyza parvicornis:* Adult. Much enlarged. Original.)

## PLATE V

Fig. 1.—*Derostenus punctiventris*, a parasite of *Agromyza parvicornis:* Adult. Much enlarged. (Original.)

Fig. 2.—*Opius utahensis*, a parasite of *Agromyza parvicornis:* Adult. Much enlarged. (Original.)



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# COLORATION OF THE SEED COAT OF COWPEAS

By Albert Mann,

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## INTRODUCTION

The following study of the seed coat of various cultivated cowpeas (*Vigna sinensis*) was made because the great diversity in color schemes and kinds of pigment in these seeds seems to have a direct bearing on problems of heredity, the pigmentation being to a large extent a basis for distinguishing one variety from another. It therefore seemed desirable that a clear understanding of the morphology of the seed coat and the way in which these pigments are arranged in its various layers should be obtained, in order to discover whether there are any facts bearing on problems of heredity, outside of the mere facts of the different color arrangements themselves. It also seemed not unlikely that such a study might prove that colors optically alike are in some cases different as to the material of the pigment and the place of its deposit.

## METHODS OF PREPARATION

The best methods for the study of the cowpea pigmentation were found to be as follows:

The seed coats were removed from dry cowpeas in flakes as large as possible and were then cut transversely in pith, the sections made by hand being as thin as possible. It was found that to embed the seed coats for microtome sectioning necessitated subjecting them to water if the freezing process was used or to various solvents if celloidin or paraffin was used. Both methods resulted in dissolving the pigments to some extent and thereby causing them to appear in parts of the seed coat where they did not normally belong. The dry method of cutting avoided this difficulty. The sections were mounted dry under ⅞-inch square cover glasses, held in place by a drop of paraffin on either side of the glass. By this method the sections may be examined in the dry state and closely watched when water, various reagents, or stains are being applied, so that facts as to solubility, chemical reaction, etc., may be accurately noted. Such sections, held down by an immovable cover glass, are also ideal for high-power examination. If necessary, they may be also readily preserved for future study. Sections tangential to the surface of the seed coat were also made, but, aside from throwing light

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upon the structure of the cells in the various tissues, it was found that they were not so useful for an examination of the pigments and their distribution as sections made transverse to the seed coat or, in other words, perpendicular to the surface of the cowpea.

A large number of reagents were experimented with, but eventually it was found that those of practical use were extremely few—namely, distilled water, alcohol, ether, chloroform, xylol, solutions of caustic soda and caustic potash (the 1 per cent solutions being of greatest service), dilute hydrochloric acid, normal Fehling's solution, saturated aqueous solution of chloral hydrate, peroxid of hydrogen solution, and several stains, the most satisfactory being a 50 per cent alcohol solution of diamond fuchsin and a 10 per cent aqueous solution of pyronin. As above stated, these were used upon sections mounted dry under the cover glass, the various liquids being drawn through by means of triangular pieces of blotting paper placed at the opposite side of the cover glass. The length of treatment varied under different circumstances from a few seconds to 24 hours. However, most of the reactions that were significant were obtained within a few minutes, so that study could be rapidly carried on.

As the problems in mind had to do with the differences in the color schemes of cowpeas as a whole, only a general examination was made to learn in what respect different areas of the seed coat were differently pigmented. It became evident that although the pigment intensification varied in different areas of the seed coat, a general idea of the color scheme could best be found by studying sections taken from the side of the seed. The greater intensity of color around the hilum was found to be merely due to a larger quantity of the same pigments as those present on the side of the seed, and very frequently this heavier pigmentation proved to be a disadvantage, as, in the case of dark colors, they frequently obscured less intense pigments easily detected in sections made where coloration was not so dense. The only case where the pigmentation near the hilum was particularly worth studying was in those varieties where colors on the general surface were lacking—namely, in the cream-white and pure-white varieties. In this class the varieties that have a more or less intense pigmentation around the hilum give some information as to the tendency of general pigmentation that such a variety might be expected to show were the whole of the seed coat colored in the usual way.

## MORPHOLOGY OF THE SEED COAT

Before discussing the coloration schemes in different varieties of the cowpea it is necessary to describe the structure of the seed coat, especially as seen in transverse sections. Such sections show that it may be divided into three layers. The outer layer is a single row of elongated palisade cells, with their long axis perpendicular to the surface of the seed

(fig. 1, *a*). Beneath this is a second layer, a single cell deep. Its cells are relatively cubical and have thick walls, but one horizontal diameter is slightly longer than the other. They are described by some authors as of hour-glass shape, a rather fanciful resemblance. They may be

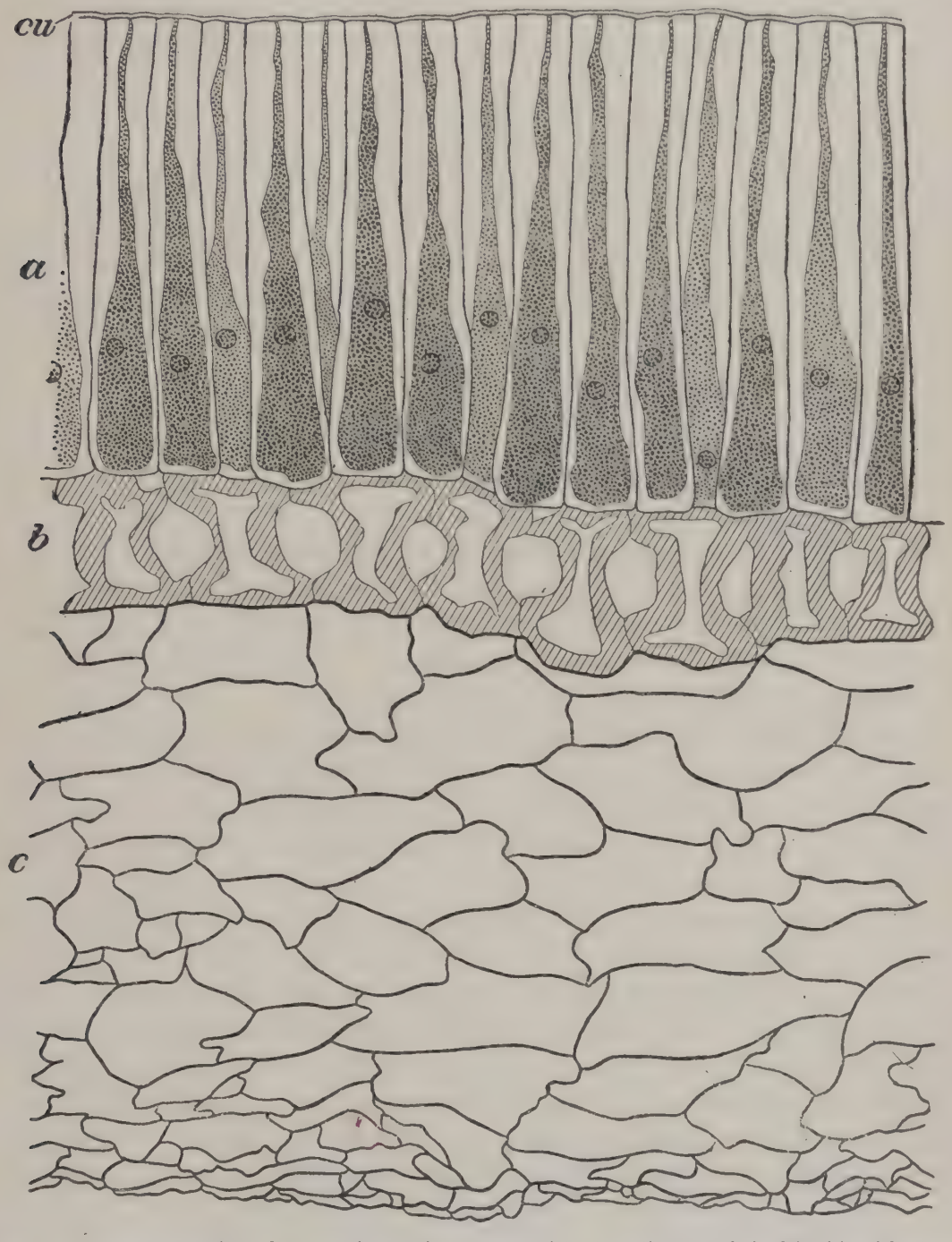



FIG. 1.—Transverse section of the seed coat of a cowpea with the cells expanded with chloral hydrate so as to show the structure of the three layers: *cu*, Cuticle; *a*, palisade layer; *b*, middle or hour-glass layer; *c*, basal-color layer.

said to lie at right angles to those of the palisade layer (fig. 1, *b*). Beneath this second layer is a comparatively thick layer from 10 to 20 cells deep, the cells being larger than those of the second layer and with relatively thin walls. These also lie parallel to the surface—that is, are hori-

zontal. They are considerably longer than broad and are, as a rule, so arranged that their longer horizontal axis is at right angles to the slightly longer horizontal axis of the cells of the second layer (fig. 1, *c*). One of the advantages of this arrangement may be to give tensile strength to the seed coat.

The middle layer of relatively cubical cells plays no rôle whatever in the pigmentation of the cowpea. Its cells are practically empty, only such residue of organic matter being present as would necessarily be found there. It is possible that there is some effect upon the coloration due to the included air which fills these cells and which in fresh-mounted sections always appears like a black band through the section; but, as the resulting color of the seed coat is made up from the different factors taken vertically, this single-celled empty layer between the palisade and the basal layers must have extremely little influence upon the color.

## PIGMENTATION OF THE BASAL-COLOR LAYER

The third or inner layer is more or less filled with a pigment, which is the same in all the cowpeas examined, and for that reason the writer calls this the basal-color layer. The pigment is a melanin-like substance,[1] ranging from a pale-straw color to a deep orange or heavy buff. As a rule, it is massed in granular particles in the lower part of the layer. In some cases the upper cells contain the larger amount of pigment, and in a few instances it is evenly distributed throughout all the cells of the basal layer. In some cowpeas, and especially in those that have a heavy basal color, the pigment completely fills a large part of the cells and is then seen to be crystalline. In such cases the color is a deep orange or sometimes even a copper red. No trace in any instance was found of any other pigment in this basal layer. Anthocyanin tests failed in every case to give a reaction.

No attempt is made in this paper to discuss the cell contents of the seed coat, outside of those substances which are directly concerned in producing the color schemes found to exist in ripe cowpeas. How the various pigments arise in the growing cells and what are the mechanical principles back of their predetermined distribution in the different varieties are questions of great cytological interest, but not important for the subject in hand. It may, however, be worth while to mention here one substance which is very generally associated with the different pigments—namely, tannin. Tests with such reagents as ferric chlorid, ferric acetate, potassium bromate, osmic acid followed by hydrogen

[1] The applying of the term "melanin" to any plant pigment has been criticized. (*See* Gortner, R. A. The misuse of the term "melanin." Science, n. s., v. 36, no. 915, p. 52–53. 1912.) Although Mr. Gortner, so far as the writer knows, has not sufficient ground to warrant his exclusion of this term from plant nomenclature, seeing that the statement that it never occurs in plants is unproved, the writer agrees that its use here is open to criticism, and has therefore substituted "a melanin-like pigment" because no advantage can be found in employing Osborn's term "humin," favored by Gortner, the boundaries of this term being at present as vague and unsettled as those of melanin.

peroxid, or any of the alkaline carbonates demonstrate that one or more of the half-dozen tannins known to exist in plants are present in appreciable quantity in all the pigmented cells of the cowpea. But the optical color effect of this tannin is too small to need any attention here. Whatever rôle tannins may play in producing the pigments deposited in the cells,[1] their chief service in the mature seed coat is undoubtedly protective.

## PIGMENTATION OF THE PALISADE LAYER

The upper or palisade layer plays a most important part in the pigmentation of the seed coat. As a rule, the cells are from 6 to 10 times as long as broad, with the cell cavity greatly enlarged at the lower or

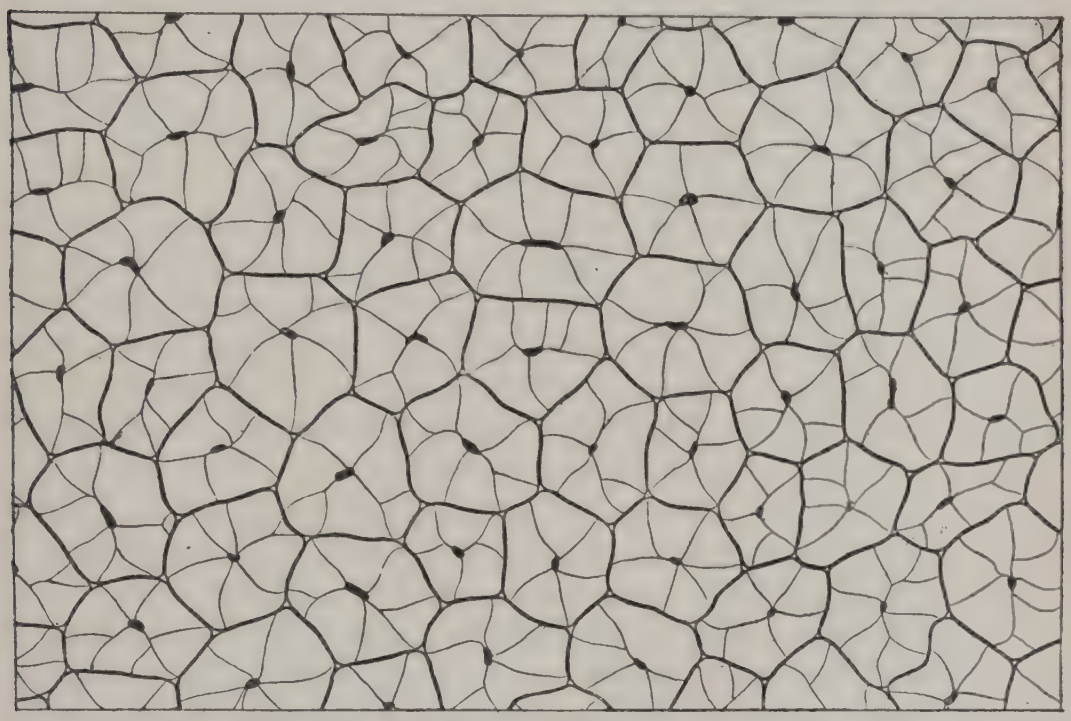


FIG. 2.—Outer surface of the seed coat of a cowpea, showing the upper ends of the palisade cells.

inner end, and gradually tapering upward to a mere thread at the upper or outer end of the cell (Pl. VI, fig. 1, *a*, and text fig. 1, *a*). From the central cavity, however, there radiate out into the gradually thickening walls from three to six vertical clefts, each reaching or nearly reaching the cell wall, thereby affording intercommunication between the cells. The result of this is that, looking on the cowpea seed coat from above, these cells appear to have at their outer end a stellate cavity (fig. 2). In focusing downward on the palisade cells these clefts gradually decrease by the widening of the central cell cavity until at or near the base the

[1] See an article on anthocyanin-forming bodies by Ioannes Politis, entitled "Sopra speciali corpi cellulari che formano Antocianine," published in Atti, R. Accad. Lincei, s. 5, Rend., Cl. Sci. Fis., Mat., e Nat., v. 20, sem. 1, p. 828–834, 1911. Politis here claims to have proved that anthocyanin is produced by certain special organs, cyanoplastids, in the composition of which tannin is a chief ingredient.

cavity occupies almost the entire width of the cell. The walls are of exceedingly dense cellulose. An outside cuticular sheath in rare cases is found covering the upper or outer surface of the palisade layer, but in most instances it is lacking (Pl. VI, fig. 1, *cu*), and the narrowed cavity of the cell seems to either reach or almost reach the outer surface of the seed coat. That there often is an actual aperture at the upper end of the cell is easily demonstrated; for when stains or colored reagents are used, it is easy to trace the rapid inflow of the liquid through these narrow, threadlike extensions of the cell cavity downward into the larger area at the lower end. Air bubbles are also seen to be pushed forward by the inflowing liquid and to pass downward into the large basal cavity. The bearing of this fact on the absorption of moisture necessary for the germinating of the seed is evident.

The normal form of the palisade cells in some varieties is strangely modified, the cell walls being very irregular. The taper of the cell cavity in such cases is imperfect, and after suddenly narrowing from the wide basal portion to a mere thread it again expands toward the outer end into a sort of mushroom-shaped enlargement. The cells themselves are also greatly contorted in outline and are sometimes spirally twisted upon their long axis, so that a true longitudinal section of these cells, such as is usually obtained in a transverse section of the seed coat, is quite impossible. Sometimes they are more or less intertwined. These distortions, as will be pointed out, are associated with certain color schemes and are quite characteristic of certain varieties.

There are two classes of pigment found in the palisade cells: First, a melanin-like pigment, identical in all its reactions and similar in its color to the pigment referred to in the lower of the three layers—namely, the basal-color layer. In some instances this is present in all the palisade cells, thereby supplementing and intensifying the basal color of the seed coat. In most cases it is confined to small groups of cells interspersed among larger or smaller areas of the palisade layer destitute of this pigment. According as this pigmentation is uniform or irregular in its deposit the basal color of the seed coat is uniform or mottled.

More frequent than this melanin-like pigment in the palisade layer are various anthocyanin pigments. These also may fill uniformly all the palisade cells or may be variously grouped and interspersed with colorless cells, thereby giving rise to the very diverse color schemes characteristic of different varieties of cowpeas. The anthocyanin pigments are practically of two kinds: First, an acid-reacting anthocyanin, ranging in color from a decided rose red to a strong purple; and, second, an alkaline-reacting anthocyanin, uniformly of a deep indigo blue, but which in mass often appears as dead black.

In many instances only one of these phases of anthocyanin pigment is discoverable in the seed coat of a given variety; and according as it fills

uniformly the cells of the palisade layer or is irregularly deposited, we have modifications in the color of the seed coat, giving rise to various forms of speckling, blotching, marbling, or monochrome coloration. In many cowpeas both the alkaline-reacting and the acid-reacting anthocyanin are present. As a rule, they are deposited in separate cells, but in a great many cases they are to be found in the same cell. When this latter is the case, the alkaline-reacting anthocyanin always occupies the lower half or third of the cell—that is, the part where the cavity is largest—and is collected in dense granular masses of a deep indigo blue. The rose-colored anthocyanin usually occupies the upper portion of the cell or occasionally fills more or less the entire cavity. The finding of both alkaline and acid reacting anthocyanin in the same cell is in harmony with a well-established cytological condition, namely, that one end of a cell may give an acid reaction while the other gives an alkaline one.

That these two phases of anthocyanin pigment are probably the same material is easily demonstrated. When thin transverse sections are suffused with neutral distilled water, the rose or purplish anthocyanin generally found in the upper end of the cell quickly diffuses into the surrounding liquid, thereby rendering more visible any alkaline indigo-blue anthocyanin which may occupy the lower portion of the cell. This latter, although also soluble in water, is very much slower in dissolving, taking several hours to disappear. Moreover, if such sections, instead of being treated with distilled water, are treated with a weak alkaline solution, such as a 1 per cent solution of caustic potash, both phases of anthocyanin undergo the same reactions. The rose-colored anthocyanin is immediately changed into an intense blue, and this, together with the indigo-blue anthocyanin, slowly passes through different shades of blue, green, greenish yellow, pale yellow, and finally is bleached and disappears. If, on the other hand, a weak solution of an acid is used, such as a 1 per cent solution of hydrochloric acid, both phases of anthocyanin again undergo the same reaction. The indigo blue immediately changes to an intense rose red and rapidly diffuses in the surrounding liquid. The tints assumed by these two phases of anthocyanin pigment are so perfectly identical with all the reagents that have been tried that it is fair to assume that we have essentially the same material in both cases, but in the one instance in an acid state and in the other in an alkaline state.

It will be seen that in all pigmentations of the palisade layer the colors are superimposed upon the underlying basal color of melanin-like pigment. It is by means of this palisade layer, therefore, that we secure the great diversity in color schemes characteristic of the cowpea. If the general pale-buff or orange-brown basal color is modified by even deposits of melanin-like pigment in the palisade layer, an intensification of the basal color is obtained, which sometimes amounts to a copper

red or dull reddish brown, uniformly spread over the seed coat. If the palisade layer contains uniformly anthocyanin in its cells, the basal color is obscured or modified by this superimposed pigment and assumes a blue or black or purple tint. If the palisade cells are irregularly pigmented, all of the modifications in marbling, speckling, and streaking which serve to distinguish the different varieties of cultivated cowpeas are found (Pl. VI, fig. 2). It may therefore be said in general that the diversification in color is principally brought about by the deposit in the palisade cells alone of the pigments above mentioned and that the different tints of color tones are the result of the various combinations of pigment already mentioned.

## SEEDS DESTITUTE OF PIGMENTATION

Some reference should be made to such cowpeas as are more or less destitute of pigmentation. The writer examined only one cowpea the entire seed coat of which gives no evidence of pigment deposit; but among the large number of white and cream-white cowpeas there are probably several others quite destitute of pigmentation—that is, true albinos. In fact, four other varieties of white or cream-white cowpeas were examined in which the maximum quantity of pigment was so minute as to make its detection quite difficult, and certain individuals of these varieties, after the most painstaking tests, left the question in doubt as to whether or not even a trace of pigment was present. They were Nos. 212–2–11, 212–6–8, 213–2–4, 214–3–2Re. From the standpoint of coloration, therefore, these pure white and cream-white varieties may all be safely considered as albinos. The strict albino examined was No. 0632. Most careful testing failed to disclose any coloration in the palisade layer or in the basal-color layer, long treatment with various reagents resulting merely in such faint tints as would be secured by reactions on the normal cell contents, such as cytoplasm and nuclear substances. With the exception of these albinos, all the cowpeas examined have more or less pigment deposited in the basal-color layer, and, as before stated, this is of a melanin-like character. When the palisade layer is destitute of pigment, uniform tinted cowpeas are obtained, ranging from a cream-white seed coat, where the amount of pigment in the basal-color layer is very small, to a strong buff or even red brown, where the amount is greater.

After this general consideration of the structure of the seed coat and of the various ways in which its color layers are pigmented, the chief varieties of cultivated cowpeas may be described individually. The varieties here enumerated represent in a general way all the known color schemes found in cowpeas. They were chosen at the suggestion of Prof. W. J. Spillman, of the Bureau of Plant Industry, who proposed this line of investigation, these types being those used by him in connection with certain studies in hybridization.

## CLASSIFICATION OF COLOR FACTORS IN COWPEAS

For convenience of reference, the foregoing factors of coloration in cowpeas may be classified as follows:

I. Basal-color layer.
  *a*. Devoid of pigment (white).
  *b*. With melanin-like pigment (buff to brown).
II. Palisade layer.
  A. Solid colors.
    *a*. Melanin-like pigments.
      *a'*. Buff, clay, etc.
      *b'*. Coffee, brown, etc.
    *b*. Anthocyanin pigments.
      *a'*. Red (acid state).
      *b'*. Blue (alkaline state).
      *c'*. Purple (combination of *a'* and *b'*).
      *d'*. Black (intensification of *b'* or *c'*).
  B. Variegated colors.
    *a*. Marbling.
      *a'*. Affects melanin-like pigments only. Whippoorwill type.
    *b*. Speckling.
      *a'*. Deep-blue anthocyanin in groups of cells, groups widely scattered. Taylor type.
      *b'*. Same, but groups more plentiful. New Era type.
    *c*. Marbling and speckling.
  C. Eyed.
    *a*. Watson type of eye. Margin of eye indefinite.
    *b*. Holstein type of eye. Seed of eye indefinite.
    *c*. Narrow eye. Narrow patch of color about the hilum indefinite at lower end (micropylar).
    *d*. Small eye, due to presence of both *a* and *b*.
    *e*. Very small eye, due to the presence of both *a* and *c*.
  D. Dilute colors.
    Characterized by individual unpigmented cells scattered among pigmented cells over entire seed coat.

## CLASSIFICATION BASED ON DISTRIBUTION AND KINDS OF PIGMENTS

For convenience in grouping, the writer has divided the different varieties into four classes: (1) Those with or without a pigment in the basal-color layer, but none in the palisade layer; (2) those in which there is a pigment in the basal-color layer and anthocyanin only in the palisade layer; (3) those in which there is pigment in the basal-color layer and a melanin-like pigment only in the palisade layer; and (4) those in which there is a pigment in the basal-color layer and both anthocyanin and a melanin-like pigment in the palisade layer.

### I.—COWPEAS HAVING NO PIGMENT IN THE PALISADE LAYER

Under the first division I have found, as previously stated, one cowpea, No. 0632, which is an extreme type of albinism. Here both the palisade and basal-color layers are destitute of all pigmentation. The

writer has not been able to discover the parentage of this variety. The reasons for its white color are two: First, the palisade cells are practically destitute of contents. Such residuary amount of cytoplasm as is present occupies a very minute part in the cell cavity and generally its upper third, instead of being in the lower end, as is usually the case. Parallel with this fact the usual spindle-shaped tapering of the cell cavity is here so slight that the diameter of the lower part is hardly greater than that of the upper part. In other words, the fine hairlike extension of the cell cavity upward does not exist. Near the upper part of the cell the somewhat narrow canal cavity widens out, and it is at this point that the small residue of cytoplasm is to be found. The cells are also more loosely bound together than usual, so that intercellular spaces between them are quite frequent. The second reason for the white color is that in the basal-color layer, which in most cowpeas is colored with a dense yellow or orange-buff pigment, there is no trace of pigment present nor any pigment reaction obtainable. The seed coat is, as a whole, much thinner and weaker than in other varieties, and its permeability to external moisture should therefore be greater.

As already stated, several other cowpeas approximate this true albino in being practically colorless, but certain individuals of these varieties show a slight trace of pigmentation in the basal-color layer. These varieties, 212–2–11, 212–6–8, 213–2–4, and 214–3–2Re, have the striking irregularity in form of the palisade cells and the lack of taper in the cell cavity just described in the case of No. 0632. Special mention should also be made of a somewhat analogous case in No. 239–5–3–18. This is also a cream-colored cowpea, but has a deep purplish pigmentation around the hilum, forming an "eye." In view of this localized pigmentation it is necessary to classify this variety under Division IV, the palisade cells in the area of the "eye" having both anthocyanin and melanin-like pigment. The color scheme of this portion of the seed coat will therefore be treated under Division IV, but as the structure of the uncolored seed coat, exclusive of the "eye," shows certain curious features identical with the white forms just described the case is here given for comparison. A transverse section of the cream-white seed coat of this cowpea shows remarkable contortion in all its layers. The palisade cells have very strongly marked the abnormal shape previously mentioned, having heavy walls and being shorter than usual; their form is irregular and twisted upon its axis. The cell cavity is very broad at the base, narrows suddenly at its middle, and again broadens slightly at the upper end. The very small residue of cytoplasm is generally found located in this upper widened portion, thus corresponding to the albino, No. 0632. Here also there is no appreciable trace of pigment to be found in the palisade cells. The cells of the remaining layers of the seed coat are also much contorted and have, in general, heavier walls than normally. A minute amount of pigment is present in the

basal-color layer and is contained in widely separated cells. It gives the same reaction as the yellow melanin-like pigment usually found in this layer. It is here, however, of a very light-straw color, this being due to the minute quantity rather than to any difference in character. This hybrid is the third generation of a cross between a Watson No. 5 and a Taylor No. 14. The significance of the contorted cells here mentioned should be borne in mind in view of its parentage, as it will be a subject for discussion under a later variety.

No. 237–3–7 is in its general color cream white, often intensified into buff, or even in a few individuals distinctly brown. The color is more conspicuous about the hilum. Therefore, it should be classified and described under Division III, although in general appearance it often seems to be uncolored.

### II.—COWPEAS HAVING ONLY ANTHOCYANIN IN THE PALISADE LAYER

The second group of cowpeas is that having only anthocyanin in the palisade cells, with a melanin-like pigment always present in the basal-color layer. Nine varieties were found to have enough difference in color scheme to be separately examined. In the first, No. 243–1–5, the seed coat is a strong red, varying to purplish brown. The palisade cells are strongly pigmented with the general color of the seed coat, so that the basal-color layer, which has the usual orange-yellow pigment, probably has little part in the general coloration, being obscured by the heavy pigmentation of the palisade layer overlying it. In neutral water the palisade pigment appears as a dull rose and slowly dissolves. Various reagents show it to be anthocyanin. Possibly it is mixed with a minute trace of buff-tinted melanin-like pigment, for there seems to remain a faint suggestion of a dull-buff pigmentation in the palisade cells after the anthocyanin has been removed.

The basal-color layer is a strong orange yellow, the pigment being the melanin-like material usually found in this layer. This variety is the second generation of a cross of Red No. 4 on Whippoorwill No. 6.

No. 253–2–3B–23 is a cowpea having a general blue-black tint, due to a speckling of deep blue on a ground color of light or dark brown, the latter being more or less obscured by the darker color. In sections treated with neutral water this pigment, an anthocyanin, shows as a strong indigo blue, confined principally to the lower ends of the palisade cells. No trace of rose-red coloration was found. Decolorized sections, if stained with diamond fuchsin, show an intensity of stain in proportion to the degree in which the cells were pigmented, and it is then more clearly seen that a fair proportion of these cells, certainly more than one-half, are without this pigment. No indication of any melanin-like pigment is found in the palisade layer. With hydrogen peroxid and ferric sulphate the palisade layer is rapidly bleached, but the basal-color layer resists the

action of this powerful liquid for some time. This layer in water shows as a deep orange-buff color, due to the melanin-like pigment generally found in this layer.

The scheme of coloration is produced by the blue-black pigment above mentioned superimposed upon the strong-clay or light-coffee color found in the basal-color layer. The strong pigmentation around the hilum is identical in character with that found on the rest of the seed coat. This hybrid is the second generation of a cross between a blue Taylor No. 20 and a Red-Eye No. 26.

A very similar cowpea in general color scheme is the so-called blue Macassar No. 21299a. The seed coat ranges in various individual cowpeas from an almost purple blue to a complete black. Transverse sections of the seed coat show that the pigment, a blue black, is somewhat unevenly distributed throughout the palisade cells, although no cells seem to be absolutely destitute of it. Associated with this blue black is a small quantity of rose-colored pigment which occupies the upper part of the cell, the deep blue being uniformly found in the lower one-third. This rose-red pigment, an acid anthocyanin, is quite evenly distributed through the palisade layer, so that the inequality in the pigmentation of the seed coat, which may be detected with a hand lens, is due to the unequally deposited deep-blue, alkaline anthocyanin.

A mere suggestion of a faint buff pigment was detected in the palisade cells, presumably the melanin-like material found in this position in other cowpeas. But as the quantity, if present, is too small to play any rôle in the coloration and its presence in any quantity is unverified, it may be left out of consideration. It should also be stated that in this cowpea a test for tannin shows that an unusual quantity of some one of this group of compounds is present in the palisade cells.

The basal-color layer is strongly pigmented with the usual deep yellow melanin-like substance. The intense color superimposed upon this by the contents of the palisade layer probably prevents its having any considerable effect in the exterior coloration of the seed coat.

This variety was secured from Piracicaba, Brazil, May, 1907. There are no data on its parentage. It may be stated that in Brazil all varieties are called "macassar."

In No. 227–2–4, a black cowpea, all of the cells of the palisade layer are well filled, at least in the upper two-thirds, with a rose-red acid anthocyanin. Somewhat less than one-half contain also an extremely dense and granular indigo-blue alkaline anthocyanin. In cells destitute of the latter the rose anthocyanin fills the entire cell. Were it not for the intense coloration due to the rose anthocyanin the unequal distribution of the indigo-blue pigment would result in a blotched or speckled condition of the seed coat. The basal-color layer is a pale-straw tint or sometimes merely a cream white. The palisade cells are free from all con-

tortion or other modification of form. This hybrid is the second generation of a cross of Watson No. 5 on Coffee No. 27.

No. 228–5–4 is usually black, but shows great variability in its color scheme, ranging from uniform black into black with small irregular fawn-colored or reddish brown marbling, or a fawn and reddish brown marbled with black, or having blue-black speckles, or in rare instances the entire cowpea is a uniform fawn or light red brown, especially immature seeds. There are, therefore, three pigment elements to be considered: (1) Deep blue or black areas, (2) blue-black speckled areas, and (3) fawn to red-brown basal areas.

The deep-black areas show that this color is an intensification of a strong purple in the pigment cells, which, although it is present in all the cells of such areas, is still quite variable as to quantity, some cells being pigmented only in the extreme lower end and others through the entire cell cavity. On treatment with neutral water this color resolves into the two factors before noticed—namely, a rose-colored anthocyanin very uniformly distributed throughout this layer and an indigo-blue anthocyanin massed in the lower end of certain cells. It is therefore evident that these black areas would show a somewhat mottled condition if the excessive pigmentation did not obscure this.[1]

The second pigment element—namely, the blue-black speckling—shows a very different condition of things. This also is due to an anthocyanin deposit in the palisade cells, but it is clearly distinguishable from what was found in the solid black areas in four respects: (1) The color is always a vivid indigo blue, not a purplish blue, nor does it give a rosy diffusion in water; (2) the quantity of this blue pigment is very much greater in the cells producing the speckling than in those found in the solid black areas; (3) it always extends upward toward the top of these cells, instead of being segregated into a heavy mass at their base; (4) the cells containing this particular pigment are in small groups, not solid masses as in the black areas, but usually large enough to be seen by the unaided eye. This is the Taylor or New Era type of speckling.

The third and remaining color is that of the fawn or reddish brown basal tint, which is due to the usual melanin-like compound contained in the cells of the basal-color layer. It ranges from a faint yellow to an intense yellow or even a copper color. This variation in quantity is the cause of the difference in color of individuals, ranging from pale fawn to reddish brown.

The palisade cells are strikingly regular, straight in outline, narrow in diameter, and long. The entire seed coat is somewhat heavier than usual. The grandparents are Clay No. 17 crossed on Coffee No. 27.

[1] Prof. W. J. Spillman has informed the writer that among cultivated cowpeas these black areas occur only in hybrids having all the factors for black pigment and having also the factor for the Taylor or the New Era style of speckling, and also that such a type can not be fixed in cultivated cowpeas, although it is the normal condition in wild cowpeas.

No. 239–4–3–6 is what is known as a Holstein pattern, a cream-white basal color blotched with large masses of black. Transverse sections through the black areas show that the pigmentation scheme is similar to that already seen in the black areas of varieties above mentioned—namely, a rose-red anthocyanin filling the upper half or two-thirds of all the pigmented cells and a blue alkaline anthocyanin deposited in only a limited number of these cells. The cells destitute of this latter pigment are in small clusters of two to five and do not make up more than one-tenth of the colored areas of the pigment layer. Here, also, a mottling of the seed coat would result were it not obscured by the intense color obtained from the heavy pigmentation of this layer. In the basal-color layer we come to a variation that has not previously been observed. Portions of this layer underlying the heavily pigmented palisade cells, which give the black areas to the seed coat, are very heavily loaded with a dense yellow granular pigment, but where the basal-color layer underlies unpigmented or cream-white areas this pigment is either wholly lacking or consists of a mere trace. Where this pigment is present in large quantities it is massed in the upper cells of the layer and is a decided copper color, whereas the lower cells are, when the pigment is present at all, a pale lemon yellow.

A still more interesting departure from normal structure is found in the white areas of the palisade layer. In the black areas these cells are quite regular, both in form and in the gradual tapering of the cell cavity. But in the white or cream-white areas, although most of them are approximately regular, occasional cells, two or three together, show the strongly contorted form and the erratic spreading of the cell cavity, which were previously noticed in the albino cowpeas. In other words, in these cream-colored areas we find a duplication of the structure of the cells, as well as of color, that was discovered in certain cream-colored cowpeas previously described. Roughly estimated, 10 per cent of the cells of the white areas of this hybrid cowpea show this striking contortion in form and erratic spreading of the cell cavity. There is therefore seen to be a very strong contrast in structure, as well as in color, between the black and the white areas of this variety. This hybrid is the product of a Watson No. 5 crossed upon a Taylor No. 14, third generation.

Cowpea No. 227–5–1Re–17 has a color scheme of the Watson type. It is a pale-buff basal color, irregularly streaked with a purple black. The colored palisade cells are extremely dense with pigment, often appearing almost black. The quantity, however, varies greatly, some cells being practically pigment-free. This results in an irregular coloration of the seed coat. The pigment is an intense indigo-blue alkaline anthocyanin, with no trace of the rose-colored acid anthocyanin. There is also no melaninlike pigment in this layer. The cells are extremely irregular, the amount of contortion being greater than that of any variety previously mentioned. The cell cavity is also irregular, being

broadly flared out at the base, rapidly narrowing in the middle of the cell to a mere thread, and again broadening at the upper end. This irregularity of form should be noted in comparison with the same condition mentioned under other cowpeas belonging to this Watson type. In the basal-color layer large crystalline masses of a heavy melanin-like pigment are deposited in clustered cells along its upper stratum. Many of the cells are so scantily supplied with pigment as to appear practically colorless. The form of the cells is also more irregular than usual, showing a decided crumpling and contortion, even when expanded by means of caustic potash. This hybrid is the result of a cross of Sport No. 5 upon Coffee No. 27, third generation.

In Sport No. 5 the seed coat varies from cream white thinly spread over with purplish black spots arranged on the Watson type to individuals in which the purplish black pigment is so abundant as to give a dull purple-gray tone to the entire seed. All the cowpeas of this variety have a strong purplish black "eye." In the darker individuals the pigmented palisade cells, about one-fifth of the entire number, owe their color to an intense indigo-blue alkaline anthocyanin uniformly found in the lower third of the cells. The other four-fifths of the cells seem to be destitute of pigmentation. The somewhat purple tint of the seed coat would lead to the expectation that an acid anthocyanin would be associated with this blue anthocyanin, but no such color was discoverable. No trace of melanin-like pigment could be found in the palisade cells. Their shape is also significant. They are usually short, with thick walls, and display to a moderate degree the strange contortion in outline and twisting on their long axis referred to in the case of some other cowpeas. The basal-color layer is a pale or dirty lemon yellow.

The only difference discoverable between the light and the dark colored individuals is the greater infrequency of pigmented cells in the palisade layer of the former, there being only 1 in 20 having a trace of the blue-black anthocyanin above noted. It is also in smaller quantity. A mere hint of rose-red anthocyanin seems to be discoverable in palisade cells of the lighter individuals, but this is too uncertain to warrant a definite statement. The cells are also unusually short, thick-walled, and contorted in outline. The basal-color layer is the same as in the other form. No accurate data as to parentage of this variety were obtainable. It was originally secured from Mr. J. W. Trinkle, of Madison, Ind., but correspondence has failed to give the facts regarding its origin.

No. 220–2–2Re, another Watson type, has a pale-buff seed coat irregularly streaked with dull purple. The pigment is intensified around the hilum, producing what is known as an "eye." A minute amount of rose-red pigment is found in the upper part of the palisade cells. It quickly dissolves in water, leaving a dense mass of granular indigo pigment in the extreme basal end of the cell cavity. A

large part of the cells, however, are practically free from any color, this being the cause of the very irregular streaked appearance of the seed coat. The cells are extremely irregular in shape, which gives to a section through the palisade layer a marked unevenness of appearance. The basal-color layer has a pale dull-yellow pigment massed in its lower cells. A good number of the cells seem to be colorless. The variety is the second generation of a cross of a Watson No. 5 on a Coffee No. 16.

One other cowpea needs to be mentioned, No. 0618. It is pale buff or clay, dusted over with brown gray on the Watson pattern. It is further pigmented with very small, round, deep, purple-brown dots, similar to the color massed around the "eye." The pigmentation of the palisade cells is confined to a little over half their number, the pigment being in the lower third of the cell cavity. It is a blue alkaline anthocyanin, with little or no trace of the acid form of this pigment, although in the minute areas represented by the deep purple-brown dots of the seed coat a small amount of red acid anthocyanin seems to be present. All the palisade cells are strongly contorted. The underlying basal layer is narrow and also strongly contorted. It is largely destitute of pigment, except for segregated masses of a deep-orange color located in widely separated groups of cells in the upper portion of the layer. The parentage of this hybrid is unknown. Like No. 5, previously mentioned, it was obtained from Mr. J. W. Trinkle, of Madison, Ind., but correspondence has failed to give any data regarding its origin.

### III.—COWPEAS HAVING ONLY A MELANIN-LIKE PIGMENT IN THE PALISADE LAYER

The third class of cowpeas—namely, those in which a melanin-like pigment alone is found in the palisade layer—ranges through various shades of light brown, buff, and red.

The first of these is a pale-buff or clay-colored cowpea, No. 237–3–7. A minute quantity of melanin-like pigment was detected in the palisade cells, and that only in the case of darker specimens. The very pale buff-colored seeds show no trace of pigmentation in this layer. The effect of this minute trace of pigment on the general color scheme of the seed coat must be small. Indeed, the color is easily explained by the stronger pigmentation of the basal-color layer. This layer is a vivid brownish yellow color. All the tests for anthocyanin failed to show a trace of this prevailing pigment in any cells of this cowpea.

It should be noted that the presence of melanin-like pigment in the palisade cells is of some interest in regard to the affinities of this cowpea to others in which it is also found, its parentage being Red No. 4 crossed on Taylor No. 14, second generation from the cross.

Another practically monochrome cowpea is No. 27544, known as the Iron cowpea. It ranges from a delicate buff or clay to a strong reddish

brown with an intensification of color about the hilum. A part of the palisade cells, perhaps two-thirds to four-fifths, contains a moderate quantity of the melanin-like pigment, the remaining cells being pigmented to only an extremely slight degree. The pigment is scattered throughout the cell cavity in a fine granular condition, instead of being massed in the lower end, as is usually the case. The basal-color layer ranges from a strong yellow to a decided copper or orange color, varying in this respect according to the general coloration of the seed coat itself. It has been impossible to learn the parentage of this well-known and widely cultivated variety.

A cowpea strongly marked in what is known as the Whippoorwill pattern, made up from a basal color of a pale clay heavily marbled with a rich reddish brown, is No. 242–3–1. The palisade cells show the variation in coloration that would be expected by the marbled character of the seed coat. The strongly pigmented cells of the marbled areas are a rich reddish brown, approaching to the color found in the basal-color layer. The other cells, making up the unmarbled areas, though not actually destitute of pigmentation, contain so minute a quantity as to only slightly affect the color of the basal layer beneath it. This latter layer is of an intense copper tint, the pigment being deposited in dense masses in the upper part of the layer. A very unequal distribution of the pigment in this layer corresponds somewhat but not accurately to the unequal distribution of the pigmentation in the overlying palisade layer. No trace of anthocyanin was found in any of the cells of this cowpea. Although the optical effect in the matter of color is not involved in the presence of tannin, it may be stated that this substance is more abundant than usual in this particular cowpea. Its parentage is Clay No. 17, crossed on Whippoorwill No. 6, second generation.

No. 243–5–3 is a variety with monochrome seed coat ranging in color from a light to a very dark reddish brown. The cells of the palisade layer show a strong granular pigment of a light red, in some cases almost brick red, quite uniformly massed in the extreme lower end of these cells. In some instances the pigment is so finely divided that it is difficult to discover it except when masses of cells are superimposed upon one another. Although the seed coat gives no indication of an unequal distribution of color, the sections seem to indicate that there is a slight excess of pigment in certain groups of cells over that in cells surrounding them. The basal-color layer has a much lighter tint than that found in the cowpea last mentioned. It is a lemon-yellow color, intensified in darker individuals to a decided brassy tone. The form of the palisade cells is normal. The parentage of this cowpea is a Red No. 4 crossed on a Whippoorwill No. 6, second generation.

No. 242–5–2 has one of the two parents last mentioned and is similar in general color scheme, varying from buff to reddish brown. The palisade cells are abundantly supplied with a dull-yellow pigment, but quite vari-

able in quantity. In view of the fact that one of its parents is a Whippoorwill this unevenness of distribution of pigment in the palisade layer is significant. The cells of this layer are longer than usual and the taper of the cell cavity is somewhat sudden and blunt. There is, however, no contortion. There is no evidence of anthocyanin. The color in the basal-color layer is somewhat different in tone from that in the palisade layer, being a more vivid yellow, approaching orange; but both give reactions that indicate the pigment to be the usual melanin-like substance. Tests for tannin show that the basal-color layer is highly impregnated with this substance. The variety is a cross of Whippoorwill No. 6 on Clay No. 17.

No. 216–6–4, a light-coffee cowpea obscurely streaked, shows the basal-color layer to be a vivid yellow, while the palisade layer is buff to brown and quite variable in degree of pigmentation. The pigment in both is a strongly granulated melanin-like substance. There is no trace of anthocyanin. The cowpea is a second-generation hybrid produced by crossing Red No. 4 upon Coffee No. 16.

No. 216–1–7 is a light to dark coffee cowpea. Closely observed, the seed coat shows a slight tendency to mottling. The very decided color of the seed coat would lead one to expect a heavy pigmentation in the palisade layer, but such is not the case. It is pale reddish brown, and not only is comparatively light, but treatment with various reagents fails to produce much intensification. In the basal-color layer the pigment is far more abundant and is confined to three or four layers, where it is somewhat unevenly distributed. It seems that the deep red brown of this cowpea is due to the pale reddish brown of the palisade layer plus the intense orange yellow of the basal layer. The parents are Red No. 4 crossed upon Coffee No. 16; in other words, it is identical with those of the cowpea last mentioned, the variety examined being the second generation of this cross.

A most interesting cowpea, known as Old Man, bears the Government number 17354. It has a cream-white seed coat obscurely and faintly streaked with yellow brown. The deeper color is very strongly deposited about the hilum, so that its character can there be readily tested. Transverse sections of the seed coat show that in almost all instances the palisade cells are practically destitute of pigment. However, a minute quantity may be detected by very close observation, and it is observable that this is highly variable, even within the narrow limits just mentioned. In other words, it corresponds to the very obscure streaking of the seed coat itself. It is of a melanin-like character without any admixture of anthocyanin. The palisade structure is decidedly abnormal, its cells being much wider in proportion to their length than common, enormously contorted, and the unusual twisting upon the long axis is here carried to an extreme. The whole palisade layer is loosely put together with abundant intercellular spaces. The basal-color layer has an exceedingly meager

and pale representation of the pigment usually present. The pale-cream color of this cowpea is doubtless due to the small quantity of melanin-like pigment diffused through the basal-color layer, and the streaked and indistinct marking of the seed coat is caused by the minute quantity of the same pigment unevenly distributed in the palisade layer. It is interesting to note that the palisade cells in the neighborhood of the hilum, where the color is quite intense and forms what is known as the "eye," are very much larger than on the rest of the seed coat and almost entirely free from the contortion and twisting already mentioned. In other words, the irregularity of form seems to be directly connected with the white or cream-white character of the seed coat. This same remarkable parallel has already been noted in several other cowpeas. The basal-color layer in the neighborhood of the hilum is very heavily charged with a melanin-like pigment, but there is here a somewhat unusual arrangement in that the lower cells of this layer are of a somewhat pale lemon yellow, while the separated masses in the upper part of the layer are a deep orange or orange buff. The reactions of these two, however, are identical. No information has been obtained as to the parentage of this variety.

### IV.—COWPEAS HAVING BOTH A MELANIN-LIKE PIGMENT AND ANTHOCYANIN IN THE PALISADE LAYER

The fourth class includes all cowpeas showing both anthocyanin and melanin-like pigment in thepa lisade layer. The first one to be mentioned, No. 214–5–10, is generally described as having buff markings upon a black ground. The fact is that it is a cowpea with a strong buff basal color almost covered with large black areas. In other words, the black is superimposed upon the buff and not the buff upon the black. The two colorations of the seed coat are accompanied by a quite different condition of the palisade layer. A melanin-like substance is to be found in all pigment cells of the seed coat both in the buff and in the black areas. An acid anthocyanin is present in all the palisade cells of the black areas, but in no case in those of the buff areas. An alkaline anthocyanin is to be found in one-half to three-fourths of the palisade cells of the black areas, but in none of the cells ot the buff areas. In all cases the alkaline anthocyanin is massed in the lower end of the cell cavity and the acid anthocyanin occupies principally, if not wholly, the upper half of the cell cavity. The color produced by these two anthocyanin pigments is a more intense purple than has been found in any other cowpea, and when the rose colored acid anthocyanin is extracted, the indigo-blue or alkaline anthocyanin found in one-half to three-fourths of the cells of the black areas is larger in quantity and more vivid in color than is generally the case. In the buff areas there is evidently neither of these phases of anthocyanin. These cells are, however, pigmented with the melanin-like material found in other cowpeas. A comparison of the form of the palisade cells in the two areas is also of interest. Those in the black

areas are unusually symmetrical, so much so as to attract attention, but in the buff areas there is a slight tendency to contortion and a more unequal tapering of the cell cavity. In other words, there is a hint in these cells of the abnormality of form found in a high degree in some other cowpeas. The basal-color layer is well supplied with the usual yellow melanin-like pigment in all parts of the seed coat. The parentage of this cowpea is White No. 7 crossed upon Black No. 22, it being the second generation hybrid.

A cowpea that appears in general purplish black, but somewhat unevenly colored, is No. 201–1–2–9. A study of its seed coat makes the cause of this evident. Many of the palisade cells contain only one anthocyanin pigment—namely, a strong rose purple. This dissolves rapidly in water, leaving the cells colorless. In some cases a second color remains in the cells and proves to be minute particles of the usual melanin-like pigment. In addition to the foregoing a number of the cells contain in the lower end a strong deposit of blue alkaline anthocyanin. This is more clearly seen after the extraction of the rose-colored anthocyanin. The melanin-like pigment is unevenly distributed in the palisade layer, many of the cells being destitute of it, so that it is safe to state that in some areas of this cowpea this pigment is associated with both phases of anthocyanin while in other parts we have either the rose anthocyanin alone or the rose and the indigo-blue phases of this pigment without the presence of the melanin-like pigment. The cross producing this variety is Black No. 13 upon Blackeye No. 19, being the third generation from the cross.

Although No. 239–5–3–18 was referred to under the first division as being an essentially cream-white cowpea, the strong purple eye of this variety places it in this last division; for by making transverse sections in the neighborhood of the hilum where the pigmentation is intense we find that the palisade layer contains both the acid and the alkaline phase of anthocyanin associated with the melanin-like pigment. The rose, acid anthocyanin is quite generally present in these pigmented cells, but a large number of them, perhaps two-thirds, are destitute of alkaline anthocyanin. The basal-color layer is abundantly colored with the usual orange-yellow pigment. As stated, the palisade cells in the cream-white seed coat, which constitutes almost the entire surface of this cowpea, are unusually irregular in form. It is therefore quite interesting to see that the strongly pigmented palisade cells in the neighborhood of the hilum show no trace whatever of these irregularities. As already stated, the hybrid is the third generation of a cross between Watson No. 5 and Taylor No. 14.

A cowpea having a basal color ranging from pale buff to strong red brown and very heavily spotted with black is No. 214–6–7–2. There is seen to be a strong brassy yellow pigment in the palisade cells. The basal-color layer is usually densely filled with the same colored pigment,

but where the basal color is pale buff instead of red brown it is very deficient. The deeper tint of the basal color in some seeds is therefore due, at least in part, to a greater quantity of pigment in the basal-color layer rather than to any difference in the pigmentation of the palisade layer. This is the reverse of what is usually found in cowpeas of variable tint, their difference usually being brought about by variation in the degree of the pigmenting of the palisade cells superimposed upon a uniformly pigmented basal layer. The black areas are due to a dense blue alkaline anthocyanin confined to the lower third of the cavity of the pigmented cells and so heavily deposited that only long action by different reagents brings about the usual changes. The brassy yellow pigment also contained in the palisade cells gives the usual reactions. It is strongly granular and in unusually large quantity. It is well to note the presence in the same cells of these two forms of pigment in connection with the fact of the very slow response of the anthocyanin to the usual reagents, as this behavior will be commented upon in other cases. The form of the palisade cells is slightly irregular, but not exceedingly so, and this is confined almost entirely to the lighter portions of the seed coat. The parentage is White No. 7 crossed upon Black No. 22, second generation.

Cowpea No. 14 has a pale-buff to red-brown basal color, strongly speckled with black spots on what is known as the Taylor pattern. In individuals of the lighter basal color we find that the palisade cells in the ground-color areas are so nearly destitute of pigment that it is difficult to discover its presence. The basal-color layer is also a dirty yellowish brown instead of the stronger brown that would be expected. The areas spotted with black owe this color to an intense blue anthocyanin in the lower half of the cavity of the palisade cells, the proportion of these to uncolored cells being about as 1 to 5. The anthocyanin extends up in these cells much higher than in most cases, sometimes reaching the upper end of the cavity. In individuals having the darker ground color, the presence of melanin-like material in the palisade cells is very evident and the basal-color layer is seen to be much more strongly tinted with a strong copper-colored melanin-like pigment. In this cowpea we again find that only by long treatment will the usual reagents bring about the expected reactions on the anthocyanin. Caustic soda, hydrochloric acid, chloral hydrate, etc., are very sluggish in the changes produced, so that there seems to be an impediment in the way of their reacting upon this sensitive material. The palisade cells in both dark and light varieties are long, narrow, evenly tapered, and symmetrical. No data have been secured as to the parentage of this variety.

No. 237–3–2 also ranges from pale buff or clay to strong red brown and is speckled with black. The black color is due to a blue alkaline anthocyanin deposited as usual in the lower end of the palisade cells, and in this case also the reagents are extremely slow in producing results

upon this pigment. This fact is of interest when taken in connection with the fact found in the other cowpeas above mentioned that it is intimately associated in these cells with a very large amount of melanin-like pigment.[1] In what way the intimate mixing of these two protects the anthocyanin from the rapid effect of reagents it is impossible to say, but it seems probable that some such interference is brought about. The melanin-like pigment is coarsely granular and orange brown in color. It is also to be observed that this pigment modifies the color tone tardily secured by the reactions of various reagents. Thus, with hydrochloric acid, the blue anthocyanin does not give a rose color, but rather a deep cherry red, probably due to the mixture of the usual rose tint with the orange yellow tint of the melanin-like pigment associated with it. The speckling, which is of the Taylor type, is due to anthocyanin contained in certain palisade cells. The basal-color layer has the usual pigment. The cowpea is the product of a cross of Red No. 4 upon Taylor No. 14, second generation.

Another cowpea of a different color scheme needs mentioning, No. 243–6–1. This one ranges from pale buff to strong red brown, speckled with black, on the New Era pattern. The palisade cells which represent the ground color—that is, which are not connected with the speckling—are, as in the former case, of a dull brassy yellow. The same tint is found in the basal-color layer. From one-third to one-fifth of the palisade cells contain a deep-blue alkaline anthocyanin located in the lower end, and here again it was discovered that all the reactions normal to this pigment are greatly delayed, so that a longer period of time is needed to make the necessary tests. The melanin-like pigment is present in large quantity in all the palisade cells. This variety is the second generation of a cross of Red No. 4, crossed upon Whippoorwill No. 6.[2]

We come now to a cowpea which is probably wild. It is a *Vigna sinensis* (?), having the number 01653, and comes from Sokoto Province, Upper Nigeria, Africa. In some respects it is quite different from the cultivated cowpea. In matter of size it is from one-seventh to one-eighth the average size of cultivated varieties. Its markings are extremely interesting, in that they display on the same seed coat all of the features which are found to make up the color schemes of the cultivated cowpeas, not only all the colors but all the styles of distribution. First, there is a basal color which ranges from pale clay or buff to reddish brown; second, this is extensively blotched or marbled with deep brown red, sometimes pretty well covering the seed coat; third, there is present a fine speckling of blue-black dots scattered over the seed coat; and

[1] This intimate mixing of blue anthocyanin with a deep-tinted melanin-like pigment and the consequent resistance of the former to reagents misled the writer at first into concluding that he here had to do with a black melanin-like substance; and in some remarks before the Washington Botanical Society on May 7, 1912, the writer included such pigment with the others found in the cowpea. A report of this meeting, in Science, June 28, 1912, also contains this error, which is now corrected.

[2] Some individuals of this variety proved to have been contaminated by crossing, hence, the presence of speckling in some of its descendants.—W. J. Spillman.

fourth, there are occasional spots in the form of large roundish intensely black areas. Transverse sections show that the general structure of the seed coat is identical with that of cultivated varieties. The palisade cells are of the same general shape and are as to size in the usual proportion to the rest of the seed coat. The underlying layer of so-called hourglass cells is also the same and is, as elsewhere, empty. Beneath this is the usual basal-color layer, supplied with the regular orange or yellow melanin-like pigment. The red areas of the seed coat overlying the basal clay or buff owe their color to a strong orange or red-brown pigment in the palisade cells, identical in organization and in its reactions with the similar color in cultivated varieties; in other words, a melanin-like pigment. The fine speckling is in this case also due to an intense blue anthocyanin pigment in the lower end of certain palisade cells and it is also here associated with the melanin-like pigment mentioned under former headings, and, as in the other cases, it responds very slowly to the reaction of reagents. Furthermore, the areas represented in the seed coat by large black spots contain both red acid anthocyanin and blue alkaline anthocyanin, as is the case in the black areas of cultivated cowpeas. The complete uniformity of methods of coloration, as well as of schemes or patterns of coloration in this supposedly wild cowpea, when compared to our cultivated varieties, is of considerable interest. There is no trace of distortion or irregularity in the palisade layer. Of course, no knowledge is obtainable as to its origin. It was received from Kew Herbarium and was collected by J. M. Dalziel.

## SUMMARY

The greatly diversified color schemes of the different varieties of cowpeas may therefore be reduced to two factors: (1) An extremely uniform basal color, ranging from very pale yellow to deep copper red, but found to be in all cases due to a melanin-like pigment deposited in the basal-color layer, the differences in tint being unquestionably caused by differences in quantity rather than in character of the pigment present; and (2) a superimposition upon this basal color of variously arranged pigment areas in the palisade layer, the outer layer of the seed coat, the pigments here being of only two kinds, first, a melanin-like pigment very generally identical in color and behavior to that found in the basal layer, and, second, an anthocyanin pigment, either associated with this or found in separate cells. And further, this anthocyanin pigment may be of a red color, on account of an acid condition, thereby producing various shades of purple and rose; or it may be alkaline in character, thereby producing various shades of blue and black, and these two may be found in the same cells or in some instances in separate cells. Finally, according as only one, or more than one, or all of these pigments sometimes found in the palisade layer are actually present there, and according as they are uniformly distributed throughout its cells or are variously localized in large or small

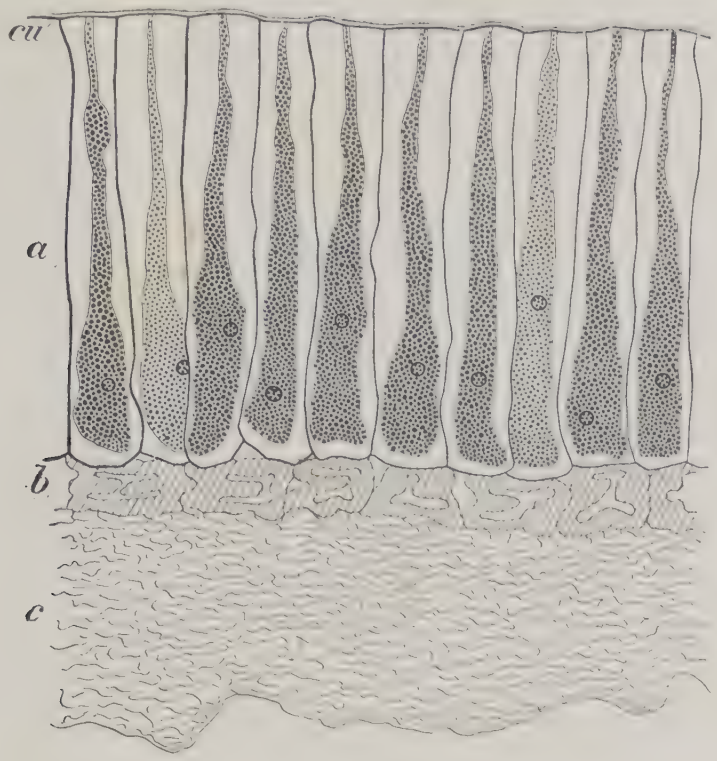
areas of its cells, do we get the remarkably diversified blotching, streaking, speckling, marbling, or monochrome colorations which characterize the different varieties of cowpeas.

A word should be said regarding the very interesting cases of distortion in the palisade cells mentioned under some of the foregoing varieties. Referring to the facts there mentioned, it will be seen that where the seed coat of the cowpea is white or cream white, as in Nos. 0362 or 17354, or where it has a certain white area, as in Holstein No. 239–4–3–6 or in No. 239–5–3–18, or even in cases where there is merely a light speckling or dusting over of this cream-white color, as in Sport No. 5, in varieties of the Watson type, as No. 227–5–1 Re–17, in certain individuals of No. 17354, and in No. 0618, the palisade cells show great distortion of outline and unevenness in the cell cavity. Furthermore, in most particolored cowpeas of strongly contrasted tints, such as Holstein No. 239–4–3–6, or the black eye in No. 239–5–3–18, or the coffee-colored eye in No. 17354, the strongly colored areas have perfectly regular, symmetrical palisade cells, while the lighter areas are more or less strongly contorted in form and irregular in the cell cavity. In other words, there is traceable in all of these cowpeas a decided correlation between the morphology of the palisade cells and the suppression of the pigments in these cells.

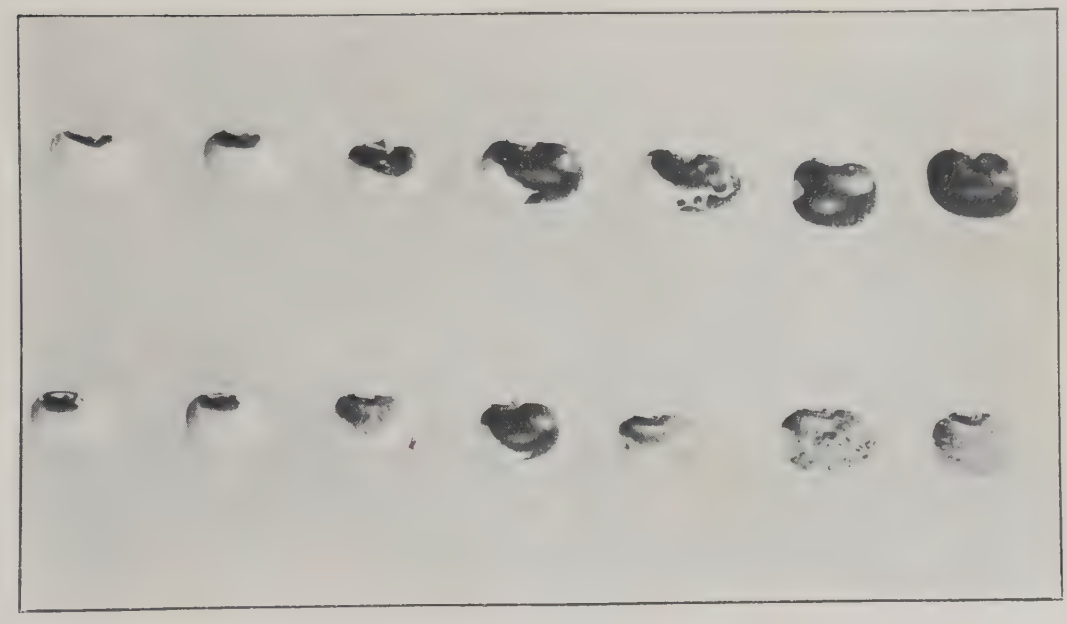
## PLATE VI

Fig. 1.—Transverse section of the seed coat of a cowpea, similar to that shown in text figure 1, but showing the relative thickness of three layers, as on the seed. The cells are not expanded with chloral hydrate. *cu*, Cuticle; *a*, palisade layer; *b*, middle or hour-glass layer; *c*, basal-color layer. Somewhat diagrammatic.

Fig. 2.—Seeds of cowpeas, showing some of the variations in the style of marking of the seed coat. Natural size.




1



2

# PRELIMINARY AND MINOR PAPERS

## EXPERIMENTS WITH APPLE LEAF-SPOT FUNGI

By JOHN W. ROBERTS,

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### INTRODUCTION

That *Sphaeropsis malorum* is the organism which causes the common leaf-spot of the apple (*Malus* spp.) so prevalent in the East and South was first shown by Scott and Rorer (1907).[1] The work of these investigators was later confirmed by the experiments of Brooks and De Meritt (1912), Lewis (1909, 1912), and others.

In various parts of the South—in Virginia, West Virginia, North Carolina, Kentucky, and Tennessee, and probably in other States—this leaf-spot, usually so prevalent in the spring, later enlarges and becomes a harboring place for various species of fungi. Such enlargements, which give to the disease the common name of "frog-eye," are usually in alternating rings or zones of brown and gray. Sometimes they form complete circles concentric with the original spot, but more often they are only half circles whose centers lie near its margin (Pl. VII, fig. 1). Around these enlargements others may be formed until perhaps one-third of the leaf is involved.

Hartley (1908) found that enlargement of spotted or injured areas could be induced to a slight extent by *Coniothyrium pirinum*. Sheldon (1908) considers the frog-eye disease in West Virginia to be due to *Illosporium malifoliorum* because of its association with the disease. Crabill (1913) gives an excellent description of the disease and expresses the belief that *Phyllosticta pirina* is very probably the factor to which the rings are due, the original spots being caused by *Sphaeropsis malorum*. The organism, however, which Crabill calls *Phyllosticta pirina*, or at least his strain No. 2 with pink spore masses, the writer believes to be *Phyllosticta limitata*. At any rate, since *Phyllosticta pirina* is a synonym of *Coniothyrium pirinum*, to recognize the two names as applying to two different fungi, as Crabill does, would be an obvious error. The fungus *Phyllosticta pirina* was transferred by Sheldon (1907) to the genus Coniothyrium on account of the color of its spores, thus making *Phyllosticta pirina* Sacc. a synonym of *Coniothyrium pirinum* (Sacc.) Sheldon. The fungus hereafter mentioned as *Phyllosticta limitata* has pink spore masses on certain culture media and on apple leaf-spots. In cultural characters it corresponds to Crabill's *Phyllosticta pirina*, strain No. 2. Its spore measurements average 7 by 4 microns.

### THE FUNGI

The writer undertook to determine whether certain leaf-spot fungi were capable of enlarging spots already formed. In the beginning the following fungi isolated from zoned spots were employed: *Coniothyrium*

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 65.

*pirinum*, *Coryneum foliicolum*, *Phyllosticta limitata*, *Monochaetia mali*, *Phomopsis mali*, and a species of Pestalozzia. Later, a species of Alternaria was also employed, to which the name *Alternaria mali* will be given, though, owing to the confused state of names and descriptions in this genus, the identification of this species with any degree of certainty is impossible. The description of the fungus and of its appearance when growing on various artificial media serves to separate it from the two or three other species which occur on the apple. Its cultural characters alone serve to separate it from one of these species, and it differs from all of them in the possession of a minutely spiny or nearly verrucose exosporium. The mycelium, as is common in the genus Alternaria, is composed of olive-tinged hyphæ rather sparingly branched. The club-shaped spores (Pl. VII, fig. 2) are for the most part muriform-septate, the transverse septa numbering usually from three to five in mature spores. The spores are 30 to 35 by 12 to 13 $\mu$ in size, with isthmi measuring 4.5 to 7 by 3 to 4 $\mu$. Its technical description is as follows:

**Alternaria mali**, sp. nov.—Hyphis fasciculatis septatis subsimplicibus vel ramulosis, griseo-olivaceis; conidiis clavatis, olivaceo-brunneis, 3–5 septato-muriformibus, ad septa constrictis, breve hispidis, 30–35×12–13 $\mu$, isthmis 4.5–7×3–4 $\mu$.

Hab. In foliis *Pyri mali*, Arlington, Virginia.

The growth of *Alternaria mali* on culture media was as follows:

BEEF AGAR +10.—Growth diffuse, grayish to nearly black at surface; aerial hyphæ nearly white and rather short.

BEEF BOUILLON.—Growth much as on beef agar, forming compact disk over surface of liquid; brownish where in contact with liquid and nearly white above.

PRUNE JUICE.—Growth forming disk over surface of medium, greenish black below; abundant flocculi of rather long aerial hyphæ, which are gray, slightly tinged with green.

PRUNE AGAR.—Growth very abundant, forming dark, nearly black crust over surface of slant, with abundance of rather long, greenish gray, flocculent aerial hyphæ, becoming darker green near the surface.

CORN-MEAL AGAR —Growth the same as on prune agar.

BEAN PODS.—Growth black where in contact with tube and dark gray where in contact with liquid. Aerial hyphæ fairly long over pod and from light gray to nearly white.

POTATO AGAR.—Growth nearly black at surface of media, with very short, scant, gray aerial hyphæ. Greatly resembles growth on beef agar.

*Alternaria mali* has several times during the last year been isolated from fruit of the apple by Mr. D. F. Fisher, of the Office of Fruit-Disease Investigations, and has been found by inoculation to cause a rapid rotting of ripe apples.

*Sphaeropsis malorum* was also used in some of the later experiments.

Lewis (1909, 1912) has shown that at least in Maine the first three of these fungi are saprophytes capable of growing and fruiting only on spots previously killed.

## EXPERIMENTS AND OBSERVATIONS ON THE FUNGUS

Inoculations were made both in greenhouse and in orchard on unsterilized leaves of susceptible varieties of apples, chiefly the Ben Davis and the York Imperial. Circular dead spots were made by touching the leaves with the heated end of a cylindrical steel rod 2 mm. in diameter. About 2,000 inoculations were made by spraying each spot with distilled water containing spores. Spots sprayed with distilled water only were considered as checks. The leaves were kept moist for periods of from one

to seven days either by inclosure in paper bags or by beginning the experiments during an extended rainy period. It soon became evident that zonate enlargements were developing from some of the check spots as well as from some of the inoculated ones, and in nearly every case the species of Alternaria appeared in culture from these enlargements. It was suspected, however, that the Alternaria was only a saprophyte and that water standing on the leaf for some time and soaking into the dead spot had thence reached the intercellular spaces of the adjacent living portion of the leaf and caused death by preventing gaseous exchange. Accordingly it was thought that if a branch of a susceptible variety with spots burned on its leaves were placed under very moist conditions the zoned areas could be induced quickly and abundantly.

On July 8 a branch of the York Imperial variety having sound leaves was selected and the cut end placed in a flask of distilled water. Without previous washing or sterilization the leaves were scorched with the heated end of the steel rod. The branch was then drenched with distilled water and placed under a bell jar partly lined with wet filter paper. Twice during the experiment the branch was taken out, and after the water on the leaves had been allowed to evaporate it was again drenched and placed under the bell jar. Under these conditions the leaves of the York Imperial apple held up as well as if they had been on the tree, there being no noticeable etiolation. In nine days 86 spots out of 111, or 77 per cent, had enlarged zones from 1 to 4 mm. in diameter, there being two or three zones to each spot. Such enlargements were then cut out from the leaves, dipped for a moment in 95 per cent alcohol, in mercuric-chlorid solution (1 to 1,000) for two minutes, in sterile water for five minutes, and then placed upon culture media. Of these cultures 17 out of 21 developed the Alternaria and 4 were sterile.

On July 19 this experiment was repeated, with the addition of two others, as follows:

EXPERIMENT 1.—Healthy leaves on branches of York Imperial apples were scorched with the heated end of a steel rod, sprinkled with distilled water, and placed under a bell jar lined with wet filter paper.

EXPERIMENT 2.—Treatment was the same as in No. 1, except that the branches were first immersed in 95 per cent alcohol for a moment, then in mercuric-chlorid solution (1 to 1,000) for two minutes, and washed in sterile water for five minutes.

EXPERIMENT 3.—One branch was treated as in No. 1 and one as in No. 2, but neither was placed under a bell jar or kept moist in any way.

The results after nine days are summarized in Table I.

TABLE I.—*Results of inoculation experiments of July 19 on branches of York Imperial apples.*

| Experiment No. | Number of burned spots. | Number of enlarged spots. | Average width of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|---|
| | | | *Mm.* | |
| 1 | 71 | 51 | 7 | 72 |
| 2 | 44 | 3 | 3 | 7 |
| 3 | 39 | 0 | 0 | 0 |

Cultures were made as in the previous experiment, except that pieces of material were left in the mercuric-chlorid solution for three minutes. Of these 9 out of 17 developed the Alternaria, 7 contained bacteria, and 1 was sterile. In these experiments it will be seen that sterilization of the leaf surfaces practically prevented the disease.

On July 28 a series of four experiments was begun. Experiments 1, 2, and 3 were performed in the same manner as Nos. 1, 2, and 3 of July 19. Experiment 4 was performed in the same manner as experiment 2, except that after the washing in sterile water the leaves were sprayed with water containing Alternaria spores from cultures made from the spot enlargements of the experiment of July 8.

In three days some of the spots were beginning to enlarge, and in seven days the results were as shown in Table II.

TABLE II.—*Results of inoculation experiments of July 28 on York Imperial apple branches.*

| Experiment No. | Number of burned spots. | Number of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|
| 1 | 101 | 66 | 65 |
| 2 | 94 | 8 | 9 |
| 3 | 41 | 0 | 0 |
| 4 | 82 | 46 | 56 |

Of 15 reisolation cultures from experiment 4, 12 developed Alternaria in pure culture and 3 contained only bacteria. Out of 18 cultures from spot enlargements of Nos. 1 and 2, 15 contained pure cultures of the Alternaria and 3 were sterile.

No counts of spot enlargements were made in the experiments of August 5, but it was noted that in those cases in which leaves were treated with alcohol and with mercuric-chlorid solution or with the mercuric-chlorid solution alone there were considerably fewer enlargements than in those untreated or those sterilized and inoculated with Alternaria spores or treated with alcohol alone. All the branches were placed under bell jars lined with wet filter paper.

The experiments of August 9 were carried on as follows:

EXPERIMENT 1.—York Imperial apple branches were placed in 95 per cent alcohol for a moment, in mercuric-chlorid solution (1 to 1,000) for three minutes, and in sterile water for five minutes. Spots were then burned on the leaves, and, after being drenched with sterile water, the branches with their cut ends in a flask of water were placed under a bell jar lined with wet filter paper.

EXPERIMENT 2.—Same as experiment 1 except that treatment with mercuric-chlorid solution was omitted.

EXPERIMENT 3.—Same as experiment 1 except that immersion in alcohol was omitted.

EXPERIMENT 4.—Treatment was the same as in experiment 1, but in addition the leaves were sprayed with sterile water containing spores of Alternaria isolated from enlarged spots of experiment of July 8.

EXPERIMENT 5.—Spots were made with a heated rod, as in the other experiments, and the leaves drenched with sterile water. The branches were then placed under a bell jar lined with wet filter paper, as in the preceding experiments. The results are given in Table III.

TABLE III.—*Results of inoculation experiments of August 9 on York Imperial apple branches.*

| Experiment No. | Number of burned spots. | Width of enlargements. | Number of enlarged spots. | | | Percentage of enlarged spots. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Aug. 14. | Aug. 16. | Aug. 18. | Aug. 14. | Aug. 16. | Aug. 18. |
| | | *Mm.* | | | | | | |
| 1 | 216 | 2 to 4 | 15 | 15 | 15 | 7 | 7 | 7 |
| 2 | 162 | 2 to 4 | 9 | 40 | 77 | 6 | 25 | 48 |
| 3 | 215 | 2 to 4 | 10 | 17 | 26 | 5 | 8 | 12 |
| 4 | 192 | 2 to 4 | 81 | 81 | 162 | 42 | 42 | 84 |
| 5 | 105 | 2 to 4 | 38 | 38 | 61 | 36 | 36 | 58 |

Cultures were made from enlarged portions of spots from experiments 2, 4, and 5. In each case marginal parts of the dead tissue were cut out, placed for a moment in 95 per cent alcohol, then in mercuric-chlorid solution (1 to 1,000) for three minutes, and in sterile water for five minutes. Of 11 cultures from experiment 2, 6 developed

Alternaria, 3 contained bacteria only, and 2 were sterile. Out of a total of 15 cultures from spot enlargements of experiment 4, 11 contained Alternaria and 4 developed bacteria. Out of a total of 12 cultures from experiment 5, 11 contained Alternaria and 1 developed bacteria.

On August 19 the following experiments were begun:

The material used in experiments 1, 2, 3, and 4 was sterilized as in No. 1 of the series of August 9. Circular spots were burned on all leaves, as in previous experiments. Nos. 1, 2, and 3 were sprayed with distilled water containing spores of *Coniothyrium pirinum*, *Phyllosticta limitata*, and *Alternaria* sp., respectively. Experiment 4 corresponded to No. 1 in the experiments of August 9 and was regarded as a check. The material used in experiment 5 was not sterilized nor inoculated, but received a thorough drenching. The branches with their cut ends in flasks of distilled water were then placed under bell jars lined with wet filter paper. The results are given in Table IV.

TABLE IV.—*Results of inoculation experiments of August 19 on York Imperial and Ben Davis apple branches.*

YORK IMPERIAL.

| Experiment No. | Number of burned spots. | Number of enlarged spots. | Average width of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|---|
| | | | *Mm.* | |
| 1 | 86 | 12 | 1.5 | 14 |
| 2 | 69 | 7 | 1.5 | 10 |
| 3 | 81 | 40 | 3 | 49 |
| 4 | 63 | 3 | 3 | 5 |
| 5 | 32 | 23 | 3 | 72 |

BEN DAVIS.

| | | | | |
|---|---|---|---|---|
| 1 | 32 | 12 | 2 | 38 |
| 2 | 32 | 12 | 1.5 | 38 |
| 3 | 31 | 30 | 3 | 97 |
| 4 | 30 | 1 | 3 | 3 |
| 5 | 43 | 25 | 2 | 58 |

Many of the enlargements in experiments 1 and 2 were somewhat doubtful ones, especially those on Ben Davis; in these two experiments the fungi fruited on practically every spot, but no fruits were found on any of the enlarged portions. Fruits would appear up to and in fact were most frequently found at the very margins of the original spots, but in no case did they occur on the newly formed parts. This agrees with the observations of Hartley (1908), who found the margins of spots to be the favorite fruiting places of *Coniothyrium pirinum*.

In cultures made as in previous experiments, material from experiment 1 produced 6 growths of the Alternaria and 2 of bacteria, while the remaining 1 was sterile; of 7 cultures from experiment 2, 2 developed Phyllosticta, 1 contained bacteria, and 4 were sterile. Out of 8 cultures from experiment 3, 6 developed Alternaria and 2 contained bacteria. Out of 15 cultures from experiments 4 and 5, 11 developed Alternaria, 2 contained bacteria, and 2 were sterile.

Experiments begun August 30 were carried on as follows:

Experiments 1, 2, 3, and 4 were performed in the same manner as those of August 19. The leaves in experiment 5 received the same treatment as those in experiment 4, but in addition they were sprayed with water containing spores of *Phomopsis mali*. As before, the branches were placed under bell jars lined with wet filter paper. The results are given in Table V.

TABLE V.—*Results of inoculation experiments of August 30 on York Imperial and Ben Davis apple branches.*

YORK IMPERIAL.

| Experiment No. | Number of burned spots. | Number of enlarged spots. | Average width of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|---|
| | | | *Mm.* | |
| 1 | 115 | 13 | 1 | 11 |
| 2 | 93 | 2 | 1 | 2 |
| 3 | 102 | 19 | 1.5 | 19 |
| 4 | 65 | 0 | 0 | 0 |
| 5 | 117 | 2 | 1 | 2 |

BEN DAVIS.

| | | | | |
|---|---|---|---|---|
| 1 | 50 | 18 | 1.5 | 36 |
| 2 | 29 | 0 | 0 | 0 |
| 3 | 34 | 14 | 3 | 41 |
| 4 | 51 | 6 | 2 | 12 |
| 5 | 43 | 2 | 2 | 5 |

Hyphæ and spores of Alternaria were found on both of the spot enlargements of York Imperial apple experiment 5.

The experiments of September 3 were carried on in an orchard at Arlington, Va. The trees selected were 10-year-old York Imperials which were entirely free from foliage diseases. These trees were planted quite closely together, so that certain portions were protected from direct sunlight during the afternoon. Leaves partly shaded in this way were selected as the most favorable for successful inoculation.

EXPERIMENT 1.—The leaves were spotted with a heated rod, as in previous experiments, and without previous sterilization were thoroughly sprayed with distilled water containing thick masses of spores of Alternaria obtained from cultures from the spot enlargements in the experiments of July 19.

EXPERIMENT 2.—The leaves were spotted as in No. 1 and without sterilization were sprayed with distilled water.

The weather during September was quite dry, but rains occurred with great frequency during October.

The results on October 22 are shown in Table VI.

TABLE VI.—*Results of inoculation experiments of September 3 on York Imperial apple branches.*

| Experiment No. | Number of burned spots. | Number of enlarged spots. | Average width of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|---|
| | | | *Mm.* | |
| 1 | 127 | 71 | 1 | 56 |
| 2 | 72 | 16 | 1 | 22 |

Cultures from the spot enlargements induced in both these experiments contained Alternaria in nearly every case.

The experiments of September 9 were carried on in the laboratory. From a well-kept orchard York Imperial branches with perfect leaves were selected and the cut ends placed in a flask of distilled water. The material used in all five experiments

was sterilized by treatment with alcohol and with mercuric-chlorid solution and the leaves were burned with a heated rod, as in previous experiments. In experiments 1 to 4 the leaves were sprayed with distilled water containing, respectively, spores of *Coniothyrium pirinum*, *Sphaeropsis malorum*, *Alternaria* sp. from cultures made from spot enlargements in experiment 4 of July 28, and *Coryneum foliicolum*. In experiment 5 the leaves were sprayed with sterile water only and considered as checks. All the branches were then placed under bell jars lined with wet filter paper. Under these conditions the leaves retained their color and turgor for 10 days and appeared to be as healthy as if they had been left on the tree. The results are given in Table VII.

TABLE VII.—*Results of inoculation experiments of September 9 on York Imperial apple branches.*

| Experiment No. | Number of burned spots. | Number of enlarged spots. | | Average width of enlargements. | | Percentage of enlarged spots. | |
|---|---|---|---|---|---|---|---|
| | | Sept. 13. | Sept. 15. | Sept. 13. | Sept. 15. | Sept. 13. | Sept. 15. |
| | | | | *Mm.* | *Mm.* | | |
| 1 | 101 | 15 | 53 | 1 | 1 | 15 | 52 |
| 2 | 94 | 0 | 11 | 0 | 1 | 0 | 12 |
| 3 | 113 | 52 | 107 | 1 | 1.5 | 46 | 95 |
| 4 | 83 | 2 | 9 | 1 | 1.5 | 2 | 11 |
| 5 | 46 | 1 | 2 | 1 | 1 | 2 | 4 |

The enlargements in experiment 3 were slightly larger than those in the other experiments. Coniothyrium was fruiting on the original spots in experiment 1, but not on the enlargements.

Of 14 cultures from enlargements of experiment 1 made as in previous series of experiments, 6 developed the Alternaria, 1 developed Coniothyrium, 4 contained bacteria, and 3 were sterile. Of 11 from experiment 2, 5 developed bacteria, 1 contained Alternaria, and 5 were sterile. From experiment 4, out of a total of 8 cultures, 4 developed Alternaria, 3 contained bacteria, and 1 was sterile.

In the series of experiments begun on September 17, York Imperial apple branches were used, as in previous experiments. All were placed under bell jars in the laboratory. Experiments 1 and 2 were performed in the same manner as the corresponding numbers in the experiments of September 9. In experiment 3 the leaves were sprayed with distilled water containing spores of *Monochaetia mali*, and in experiment 4, the check, they were sprayed with sterile water. All leaves were sterilized, and spots were burned upon them, as in previous experiments. The results on September 23 are given in Table VIII.

TABLE VIII.—*Results of inoculation experiments of September 17 on York Imperial apple branches.*

| Experiment No. | Number of burned spots. | Number of enlarged spots. | Average width of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|---|
| | | | *Mm.* | |
| 1 | 87 | 17 | 1 | 20 |
| 2 | 167 | 32 | 2 | 19 |
| 3 | 65 | 3 | 1 | 5 |
| 4 | 53 | 10 | 2 | 19 |

Out of a total of 18 cultures from the spot enlargements of experiment 2, 17 contained Alternaria and 1 developed bacteria. In experiment 1 Coniothyrium fruits appeared on every spot, but there was none on the enlargements. As before, the favorite place

for the pycnidia of this fungus to appear was at the very edge of the original spot. In experiment 2, 7 spots, and in experiment 4, 2 spots had enlargements 4 mm. in width, with tufts of Alternaria hyphæ on their surfaces. All the enlargements were strikingly zonate.

In the experiments of September 24 the leaves of York Imperial branches received the same treatment as in the experiments of September 17, both as to sterilization and spotting. In experiments 1 to 3 the leaves were sprayed with distilled water containing spores of Phomopsis, Sphaeropsis, and Alternaria, respectively, while in No. 4, the check, they were sprayed with sterile water only. All the branches were then placed under bell jars lined with wet filter paper. The results after five days are given in Table IX. No attempts at reisolation were made.

TABLE IX.—*Results of inoculation experiment of September 24 on York Imperial apple branches.*

| Experiment No. | Number of burned spots. | Number of enlarged spots. | Average width of enlargements. | Percentage of enlarged spots. |
|---|---|---|---|---|
| | | | *Mm.* | |
| 1 | 99 | 2 | 1 | 2 |
| 2 | 71 | 3 | 1 | 4 |
| 3 | 102 | 47 | 1 | 46 |
| 4 | 51 | 4 | 1 | 8 |

It will be noticed that, owing to the natural prevalence of Alternaria on the leaves, sterilization was quite difficult. From the writer's experience in making cultures from fruit, leaves, and twigs of apples grown in the South, he believes Alternaria to be the most widely distributed fungus on the apple in that section of the country. In order to make the amount of natural infection about the same in all cases, all branches used in a series of experiments were sterilized together so that each leaf received the same treatment.

TABLE X.—*Summary of results from all experiments in which sterilized leaves were used.*

| Treatment. | Number of burned spots. | Number of enlarged spots. | Percentage of enlarged spots. |
|---|---|---|---|
| Sprayed with spores of— | | | |
| Coniothyrium | 471 | 123 | 27 |
| Sphaeropsis | 332 | 46 | 14 |
| Alternaria | 864 | 536 | 62 |
| Phomopsis | 259 | 6 | 2 |
| Coryneum | 83 | 9 | 11 |
| Monochaetia | 65 | 3 | 5 |
| Sprayed with sterile water | 928 | 62 | 7 |

The enlargements induced in each series of experiments bore a remarkable resemblance to the enlarged leaf-spot or frog-eye disease of the South. As shown in Plate VII, figure 3, the zonate effect was quite pronounced. This, as in the natural frog-eye, was due to an alternation of gray and brown coloration. Sometimes, also, the zone extended completely around the original spot, and sometimes its center lay at the margin of a spot, as is the case with frog-eye disease in nature. Alternaria was the only fungus isolated with any degree of consistency from any of these zones.

In some of the series of experiments the branches were removed from under bell jars for an hour every other day and the leaves allowed to dry. In such cases there seemed to be a rough relation between the number of such periods and the number of zones.

In one orchard in Virginia the leaves of eight York Imperial apple trees had been badly spotted through injury by sprays. Later, these spots enlarged and became typical frog-eye spots. Cultures made from these enlargements with the same precautions as previously outlined developed Alternaria in practically every case.

## CONCLUSIONS

From these experiments one is justified in concluding that under certain conditions *Alternaria mali* is able to enlarge dead spots of apple leaves and may be classed as a rather strong facultative parasite. *Coniothyrium pirinum* possesses but little power of enlarging dead areas and may be classed as a saprophyte or at best as a weak facultative parasite. *Coryneum foliicolum*, *Phyllosticta limitata*, *Monochaetia mali*, and *Phomopsis mali* are, in so far as apple leaves are concerned, purely saprophytic.

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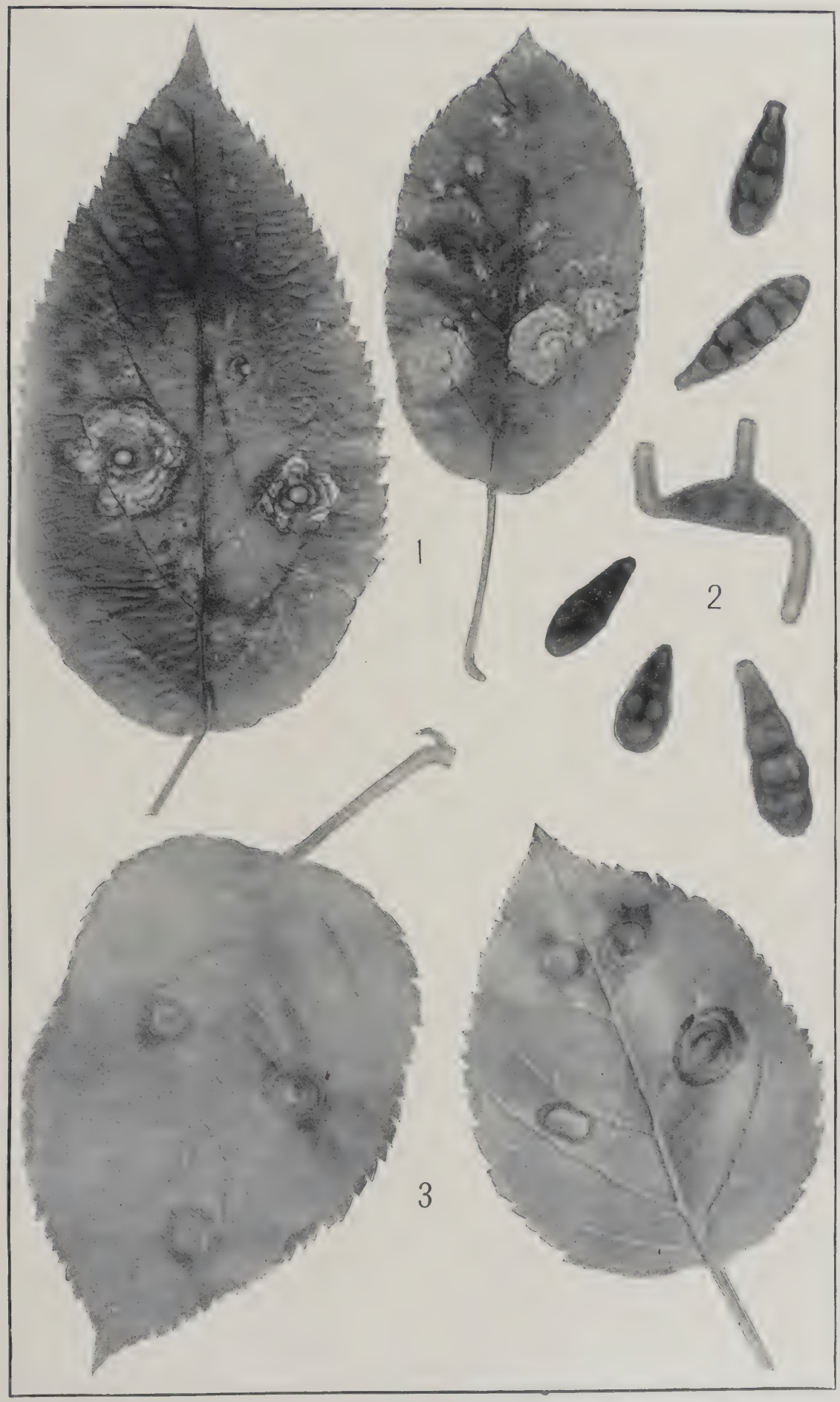
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## PLATE VII

Fig. 1.—Apple leaves from Tennessee, showing typical spots of the frog-eye disease.

Fig. 2.—Spores of *Alternaria mali*, which is capable of enlarging dead spots on apple leaves.

Fig. 3.—York Imperial apple leaves, showing spots enlarged by *Alternaria mali*. The dead centers of the spots were produced by burning with a heated rod.


1
2
3

# LONGEVITY OF PYCNOSPORES OF THE CHESTNUT-BLIGHT FUNGUS IN SOIL

By F. D. Heald and M. W. Gardner, *Agents, Investigations in Forest Pathology, Bureau of Plant Industry*

## INTRODUCTION

That "spore horns" of the blight fungus *Endothia parasitica* (Murr.) And. are dissipated by rains has been recognized since the first studies on the chestnut bark disease. That the pycnospores are washed down the tree and are responsible for reinfections has been especially noted by Collins (1912).[1] This fact was also emphasized by Metcalf and Collins (1911), as may be noted from the following quotation:

> When the spores of the fungus are present, especially in the form of threads, or "horns," they are readily washed down the branches and trunk by every rain, and are thus carried down to or toward the base of the tree.

During the winter and spring of the year 1913 investigation of the dissemination of the chestnut-blight fungus by the writers disclosed the fact that during every rain, even at seasons when spore horns were not produced, pycnospores in great numbers were washed down the trunks of diseased trees. (Heald, 1913b; Heald and Gardner, 1913a–b.) This naturally led to the question as to what became of these millions of pycnospores washed into the soil.

Several possibilities suggested themselves. One of these was that the spores might germinate in the soil water and thus be readily killed by desiccation or other unfavorable conditions. Another was that, though the spores would not germinate, their period of viability might be very short even in wet soil, or that possibly they might retain their viability until it was terminated by unfavorable conditions such as freezing or drying out of the soil.

On the other hand, it was possible that the spores might remain viable not only during the time that the soil was wet but that they might endure for extended periods such unfavorable conditions as those produced by freezing or desiccation.

As to the possibility of pycnospores germinating in soil water, the tests so far carried out with various soil extracts have yielded inconclusive results. Pycnospores will germinate in certain soil extracts and not in others, the percentage of germination ranging from 0 to 65.

Our tests on the longevity of pycnospores in sterile tap water may be cited here as having some bearing on their persistence in wet soil. The few tests made have shown that 61 to 93 per cent retain their viability for four weeks. Only one trial was made to determine their power to endure freezing in sterile tap water. A very large percentage, 96, survived a period of six days of freezing, and 10 per cent survived a second period of eight days of freezing. Further investigation along all of these lines is planned.

Regarding the resistance of pycnospores to desiccation in soil, more conclusive results have been obtained. The purpose of the tests recorded

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 75.

in the following pages was to determine the resistance of pycnospores washed into the soil in the field to desiccation in this soil under two distinct conditions: First, in the undisturbed soil in the field during the intervals between rains; and, second, during a more prolonged period of desiccation in the laboratory.

All except three of the soil samples used in these tests were secured in the experimental plat near West Chester, Pa., at the bases of diseased chestnut trees bearing certain of the "pycnospore traps" used in the analysis of rain water washed down the trunks (Heald and Gardner, 1913a–b). The results of these analyses proved that few, if any, ascospores were present in the water washed down the trees into the soil, even late in the spring after ascospore expulsion was in progress. From these data and from the time of appearance of the colonies in our cultures it is certain that the spores with which we were dealing were pycnospores and not ascospores (Heald, 1913a).

## TESTS OF LONGEVITY UNDER FIELD CONDITIONS

The first samples of soil in each series used in determining the power of resistance of the pycnospores to desiccation in soil under field conditions were taken as soon as possible after the cessation of a rain. The litter and immediate surface soil were scraped away from a small area at the base of the tree, and with a sterile knife or spoon a sample of the soil thus exposed and immediately adjacent to the base of the trunk was removed to a sterile tin receptacle for transport to the laboratory.

The dilution method with poured-plate cultures was used in ascertaining the number of viable pycnospores per gram of soil. Using sterile utensils, 1 gram of the soil was weighed out and placed in a flask containing 99 c. c. of sterile water. By crushing the soil lumps with a flamed glass rod and by agitation of the flask a thoroughly uniform suspension was obtained. From this suspension 1 c. c. was transferred with a sterile pipette to a second flask containing 99 c. c. of sterile water, and of this second dilution measured quantities (1 c. c. and fractions thereof) were placed in sterile Petri dishes and plated out in standard 3 per cent dextrose agar +10. From the number of colonies of the blight fungus developing in these cultures an estimate was obtained of the number of viable pycnospores present in the gram of soil tested.

In securing the second or third samples of soil from each location in the field the soil was taken from a point as near as possible to the exact source of the first sample and from a similar position relative to the tree. The method used in testing these subsequent samples was identical with that previously outlined, no allowance being made for loss of weight due to drying. In making a second or third test it is evident that a different individual portion of soil must be used in which the original number of spores contained would probably by no means coincide with the spore content of the previous sample from that location. Taking this fact into consideration, the discrepancies in the figures are only such as might be expected.

The period of desiccation in these field determinations was necessarily limited by the recurrence of rain, so that in series A, B, and C only one subsequent test was obtained for each location. In series D, however, a rather extended period of dry weather permitted tests to be made after relatively longer periods of desiccation. The results obtained are presented in Table I.

TABLE I.—*Longevity tests of pycnospores in soil in the field in 1913.*

[Samples taken from bases of diseased chestnut trees bearing pycnospore traps.]

SERIES A.

| Trap No. and soil sample No. | Rainfall. Date. | Rainfall. Inches. | Date of collection. | Date of cultures. | Period of drying. | Condition of soil when tested. | Number of viable spores per gram of soil. |
|---|---|---|---|---|---|---|---|
| | | | | | *Days* | | |
| Trap I: | | | | | | | |
| Sample 1 | Mar. 20 | 1.64 | Mar. 21 | Mar. 21 | 1 | Wet from rain | 1,140,000 |
| Sample 3 | | | Mar. 24 | Mar. 24 | 4 | Nearly air-dry | 47,072 |
| Trap III: | | | | | | | |
| Sample 2 | ..do | 1.64 | Mar. 21 | Mar. 21 | 1 | Wet from rain | 527,724 |
| Sample 4 | | | Mar. 24 | Mar. 24 | 4 | Nearly air-dry | 21,509 |

SERIES B.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Trap I: | | | | | | | |
| Sample 5 | Apr. 4 | .56 | Apr. 5 | Apr. 5 | ½ | Wet from rain | 3,604,000 |
| Sample 7 | | | Apr. 7 | Apr. 7 | 2½ | Damp | 7,128,000 |
| Trap III: | | | | | | | |
| Sample 6 | ..do | .56 | Apr. 5 | Apr. 5 | ½ | Wet from rain | 4,410,000 |
| Sample 8 | | | Apr. 7 | Apr. 7 | 2½ | Damp | 1,998,000 |

SERIES C.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Trap I: | | | | | | | |
| Sample 9 | Apr. 10–16. | 4.06 | Apr. 18 | Apr. 19 | 2½ | Damp from rain | 6,570,000 |
| Sample 11 | | | Apr. 21 | Apr. 22 | 5½ | Air-dry | 1,640,000 |
| Trap III: | | | | | | | |
| Sample 10 | ..do | 4.06 | Apr. 18 | Apr. 19 | 2½ | Damp from rain | 2,412,666 |
| Sample 12 | | | Apr. 21 | Apr. 22 | 5½ | Air-dry | 288,000 |

SERIES D.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Trap I: | | | | | | | |
| Sample 13 | Apr. 27–28. | 2.43 | Apr. 28 | Apr. 29 | 0 | Very wet | 1,209,500 |
| Sample 17 | | | May 5 | May 6 | 6 | Air-dry | 2,723,333 |
| Sample 21 | Apr. 29 | .11 | May 12 | May 13 | 13 | Loose, air-dry surface soil exposed to sun. | 1,077,333 |
| Trap III: | | | | | | | |
| Sample 14 | Apr. 27–28. | 2.43 | Apr. 28 | Apr. 29 | 0 | Very wet | 2,948,000 |
| Sample 18 | | | May 5 | May 6 | 6 | Air-dry | 86,000 |
| Sample 22 | Apr. 29 | .11 | May 12 | May 13 | 13 | Loose, air-dry surface soil partly shaded. | 84,000 |
| Trap IV: | | | | | | | |
| Sample 15 | Apr. 27–28. | 2.43 | Apr. 30 | May 1 | 1 | Wet from rain | 3,336,000 |
| Sample 19 | | | May 5 | May 6 | 6 | Air-dry | 3,792,000 |
| Sample 23 | Apr. 29 | .11 | May 12 | May 13 | 13 | Loose, air-dry surface soil exposed to sun. | 2,412,333 |

TABLE I.—*Longevity tests of pycnospores in soil in the field in 1913*—Continued.

| Trap No. and soil sample No. | Rainfall. Date. | Rainfall. Inches. | Date of collection. | Date of cultures. | Period of drying. | Condition of soil when tested. | Number of viable spores per gram of soil. |
|---|---|---|---|---|---|---|---|
| Trap VI: | | | | | *Days* | | |
| Sample 16. | Apr. 27–28. | 2.43 | Apr. 30 | May 1 | 1 | Wet from rain.. | 490,000 |
| Sample 20. | | | May 5 | May 6 | 6 | Air-dry..... | 1,100,000 |
| Sample 24. | Apr. 29 | .11 | May 12 | May 13 | 13 | Loose, air-dry surface soil exposed to sun. | 368,000 |

## TESTS OF LONGEVITY UNDER LABORATORY CONDITIONS

For use in determining the longevity of pycnospores dried in soil and stored in the culture room in the laboratory to secure longer periods of desiccation, samples were collected in much the same manner as has been described for the field tests. A much larger quantity of soil was taken from the bases of diseased trees after a rain and transported to the laboratory in sterile tin containers. During storage in the culture room the tin covers were replaced by layers of absorbent cotton held in place by rubber bands. Thus, ample opportunity was afforded for thorough drying of the soil.

These samples were tested as soon as possible after collection while the soil was still wet and at convenient intervals thereafter during the period of storage to ascertain the number of viable pycnospores to the gram. With some exceptions the method used in the tests was identical with that already described for the field tests. Previous to each test, however, the soil in each container was thoroughly shaken to secure as uniform a mixture as possible. As in the previous work, no allowance was made for loss of weight due to evaporation of the soil moisture. Most of this loss occurred during the first period of drying, the soil being practically air-dry and readily reducible to dust at the end of one week. By numerous trials the loss in weight due to air-drying was found to represent an average decrease of 35 to 40 per cent of the weight at the time the first test was made. A factor of 1.66⅔ might be applied to the figures given in the first or control analysis of each sample to compensate for this error.

In all of the cultures made previous to July 30, 3 per cent dextrose agar + 10 was used as the medium. In the cultures made on July 30, August 8, and August 26 chestnut-bark agar made from diseased bark was employed; in the final tests chestnut-bark agar made from healthy bark was employed. Furthermore, relatively larger portions of inoculum were used in the final tests (September 25), 1 c. c. being transferred from the first suspension to a flask containing only 9 c. c. of sterile water from which 1 c. c. and fractions thereof were plated out. The results obtained are presented in Table II.

TABLE II.—*Longevity tests of pycnospores in soil stored in culture room in 1913.*

SERIES E.

[Rainfall: Date, April 27 and 28; amount, 2.43 inches. Date of collection of soil samples, April 28. Source of soil samples: No. 13. Base of sprout bearing pycnospore trap No. I. No. 14. Base of tree bearing pycnospore trap No. III.]

| Date of test. | Number of days of drying. | Number of viable spores per gram of soil. | |
|---|---|---|---|
| | | Soil sample No. 13. | Soil sample No. 14. |
| Apr. 29 | 0 | 1,209,500 | 2,948,000 |
| May 8 | 9 | 282,000 | 240,000 |
| May 16 | 17 | 42,000 | 446,000 |
| May 23 | 24 | 62,000 | 217,142 |
| May 31 | 32 | 0 | 280,000 |
| June 6 | 38 | 205,333 | 270,000 |
| June 12 | 44 | 116,875 | 504,000 |
| June 19 | 51 | 12,666 | 216,000 |
| June 27 | 59 | 54,000 | 41,428 |
| July 3 | 65 | 11,428 | 1,272,000 |
| July 11 | 73 | 0 | 30,526 |
| July 20 | 82 | 25,333 | 31,356 |
| July 30 | 92 | 20,571 | 0 |
| Aug. 8 | 101 | 0 | 0 |
| Aug. 26 | 119 | 0 | 17,857 |
| Sept. 25 | 149 | 0 | 0 |

SERIES F.

[Rainfall: Date, Apr. 27, 28, and 29; amount, 2.54 inches. Date of collection of soil samples, Apr. 30. Source of soil samples: No. 15. Base of tree bearing pycnospore trap No. IV. No. 16. Base of tree bearing pycnospore trap No. VI.]

| Date of test. | Number of days of drying. | Number of viable spores per gram of soil. | |
|---|---|---|---|
| | | Soil sample No. 15. | Soil sample No. 16. |
| May 1 | 0 | 3,336,000 | 490,000 |
| May 8 | 7 | 820,000 | 91,875 |
| May 16 | 15 | 532,000 | 53,333 |
| May 23 | 22 | 51,428 | 144,000 |
| May 31 | 30 | 204,054 | 68,571 |
| June 6 | 36 | 96,000 | 95,143 |
| June 12 | 42 | 62,666 | 43,333 |
| June 19 | 49 | 92,000 | 70,000 |
| June 27 | 57 | 63,000 | (?) |
| July 3 | 63 | 42,857 | 0 |
| July 11 | 71 | 0 | 14,000 |
| July 20 | 80 | 18,636 | 0 |
| July 30 | 90 | 11,714 | 0 |
| Aug. 8 | 99 | 0 | 0 |
| Aug. 26 | 117 | 0 | 0 |
| Sept. 25 | 147 | 0 | 0 |

TABLE II.—*Longevity tests of pycnospores in soil stored in culture room in 1913*—Contd.

SERIES G.

[Rainfall: Date, May 16 and 17; amount, 0.74 inches. Date of collection of soil samples, May 19. Source of soil samples: Orchard of grafted Paragon trees, Martic Forge, Pa.[1] No. 17. Base of tree bearing ascospore trap No. 44. No. 18. Base of tree bearing ascospore trap No. 43. No. 19. Base of tree bearing ascospore trap No. 43.]

| Date of test. | Number of days of drying. | Number of viable spores per gram of soil. | | |
|---|---|---|---|---|
| | | Soil sample No. 17. | Soil sample No. 18. | Soil sample No. 19. |
| May 20 | 0 | 4,106,666 | 1,196,000 | 8,890,000 |
| May 27 | 7 | 3,808,000 | 925,000 | 1,840,000 |
| June 4 | 15 | 2,318,666 | 630,000 | 4,966,000 |
| June 12 | 23 | 1,351,250 | 530,000 | 4,156,666 |
| June 19 | 30 | 816,000 | 94,285 | 3,192,000 |
| June 27 | 38 | 920,000 | 116,000 | 2,240,000 |
| July 3 | 44 | 468,000 | (?) | 1,662,727 |
| July 11 | 52 | 733,333 | 30,000 | 972,571 |
| July 20 | 61 | 204,545 | 0 | 90,000 |
| July 30 | 71 | 68,148 | 46,000 | 59,259 |
| Aug. 8 | 80 | 0 | 0 | 0 |
| Aug. 26 | 98 | 0 | 0 | 5,000 |
| Sept. 25 | 128 | 0 | 0 | 0 |

[1] Collected by Mr. C. E. Taylor, formerly in the employ of the Pennsylvania Chestnut Tree Blight Commission.

SERIES H.

[Rainfall: Date, May 16 and 17; amount, 0.61 inch. Date of collection of soil samples, May 19. Source of soil samples: No. 20. Base of tree bearing pycnospore trap No. III. No. 21. Base of tree bearing pycnospore trap No. VI.]

| Date of test. | Number of days of drying. | Number of viable spores per gram of soil. | |
|---|---|---|---|
| | | Soil sample No. 20. | Soil sample No. 21. |
| May 21 | 0 | 2,832,000 | 2,640,000 |
| May 27 | 6 | 750,000 | 1,063,333 |
| June 4 | 14 | 364,000 | 571,428 |
| June 12 | 22 | 100,800 | 294,853 |
| June 19 | 29 | 26,666 | 150,857 |
| June 27 | 37 | 170,500 | 115,142 |
| July 3 | 43 | 68,181 | 140,000 |
| July 11 | 51 | 21,714 | 82,000 |
| July 20 | 60 | 15,000 | 21,176 |
| July 30 | 70 | 177,777 | 25,925 |
| Aug. 8 | 79 | 12,413 | 0 |
| Aug. 28 | 97 | 0 | 0 |
| Sept. 25 | 127 | 0 | 0 |

TABLE II.—*Longevity tests of pycnospores in soil stored in culture room in 1913*—Contd.

SERIES K.

[Rainfall: Date, May 21, 22, 23, and 24; amount, 3.24 inches. Date of collection of soil samples, May 26. Source of soil samples: No. 22. Base of tree bearing pycnospore trap No. I. No. 23. Base of tree bearing pycnospore trap No. IV. No. 24. Base of tree bearing pycnospore trap No. VI.]

| Date of test. | Number of days of drying. | Number of viable spores per gram of soil. | | |
|---|---|---|---|---|
| | | Soil sample No. 22. | Soil sample No. 23. | Soil sample No. 24. |
| May 27 | 0 | 3,760,000 | 4,480,000 | 2,270,333 |
| June 4 | 8 | 1,300,000 | 1,530,000 | 1,050,000 |
| June 12 | 16 | 38,571 | 578,000 | 270,000 |
| June 19 | 23 | 52,000 | 160,000 | 82,285 |
| June 27 | 31 | 1,611,428 | 90,000 | 206,666 |
| July 3 | 37 | 553,714 | 322,000 | 92,571 |
| July 11 | 45 | 0 | 145,454 | 0 |
| July 20 | 54 | 62,000 | 70,454 | 0 |
| July 30 | 64 | 28,000 | 0 | 11,000 |
| Aug. 8 | 73 | 0 | 0 | 0 |
| Aug. 26 | 91 | 0 | 0 | 5,000 |
| Sept. 25 | 121 | 0 | 0 | 0 |

SUMMARY.

| No. of soil sample. | Number of viable spores per gram of soil before drying. | Number of days spores remained viable (longevity limit). | Number of viable spores per gram of soil in last test showing presence of viable spores. |
|---|---|---|---|
| 13 | 1,209,500 | 92 | 20,571 |
| 14 | 2,948,000 | 119 | 17,857 |
| 15 | 3,336,000 | 90 | 11,714 |
| 16 | 490,000 | 71 | 14,000 |
| 17 | 4,106,666 | 71 | 68,148 |
| 18 | 1,196,000 | 71 | 46,000 |
| 19 | 8,890,000 | 98 | 5,000 |
| 20 | 2,832,000 | 79 | 12,413 |
| 21 | 2,640,000 | 70 | 25,925 |
| 22 | 3,760,000 | 64 | 28,000 |
| 23 | 4,480,000 | 54 | 70,454 |
| 24 | 2,270,333 | 91 | 5,000 |

## CONCLUSIONS

The results obtained in these two sets of tests lead to several conclusions. The field tests show that the pycnospores are to a considerable degree resistant to desiccation in soil in the field and that a large number may retain their viability during a period of 2 to 13 days of dry weather.

In the indoor tests, where the period of drying could be prolonged at will, more conclusive results were secured. In each sample it is seen that, with some irregularities, there is a gradual decrease in the number of viable spores as the period of desiccation is prolonged. The irregularities in the figures are due partly to the fact that from the standpoint of spore content it was impossible to secure a perfectly uniform mixture in the soil sample.

It is evident that in every case except one a large number of spores survived two months of desiccation and that in 5 out of the 12 samples not all of the spores had succumbed after three months of drying. The longevity limit varies from 54 to 119 days, the average being 81 days. At the end of periods of desiccation ranging from 121 days for some samples to 149 days for others no viable spores were found. The conclusiveness of these final tests is enhanced by the fact that relatively larger quantities of inoculative material were used in the cultures. (See summary of Table II.)

These results suggest that viable pycnospores are constantly present in the soil beneath infected trees, since each succeeding rain replenishes the supply. Under normal conditions there would hardly be a period of drought sufficiently extended to destroy all of the enormous number washed into the soil.

The fact that a large percentage of the pycnospores which are washed into the soil withstand two to three months of drying has an interesting bearing upon certain phases of the problem of dissemination of the chestnut bark disease and affords at least three possibilities for conjecture.

First, it shows that pycnospores will resist the degree of drying necessary to reduce the soil to a condition in which it might be easily pulverized and blown about as dust. This presents the possibility of wind dissemination of pycnospores dried on soil particles. Such a possibility has been suggested by Metcalf and Collins (1911), and Collins (1912). However, this contingency is rather improbable under natural forest conditions, since the soil beneath the trees is more or less protected and dries much less rapidly than in the open. On the other hand, there would be a fair probability of wind transport of pycnospores in blight-infected nurseries without ground cover or from more isolated trees, especially if the soil is exposed or the ground cover is sparse.

In the second place, a means is presented whereby pycnospores might be transported in mud dried on insects, on the feet of birds, squirrels, and other animals, and even on the shoes of man. Such might be the explanation of the presence of pycnospores found on one of the two juncos tested during the study of birds as carriers of the chestnut blight fungus (Heald and Studhalter, 1913), since the junco obtains nearly all of its food from the ground.

Finally, the results of these tests present the possibility of the transportation of pycnospores in the soil adhering to the stems and roots of chestnut nursery stock during shipment. Such nursery stock might be

uninfected and hence pass inspection as free from disease, the spores having been washed into the soil from diseased parts pruned from the specimens before shipment or from diseased plants which were adjacent to the healthy specimens in the nursery. Under such conditions it is evident that large numbers of pycnospores might retain their viability during long periods of shipment.

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# BEHAVIOR OF CUP CURRENT METERS UNDER CONDITIONS NOT COVERED BY STANDARD RATINGS

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Experience gained in taking a long series of canal measurements led the writer to believe that the conditions met in field measurements of the flow of water in open channels by the use of current meters varied so widely from those under which the meters are rated as to render inaccurate to an appreciable degree the results of field measurements computed on the basis of the ratings usually made. For the purpose of determining this point, a series of experiments was made, with the results reported here.

The meter used was the small Price meter commonly used for determining the velocity of flowing water. The usual practice in rating a current meter is to make the tests for all velocities with the meter at the same distance beneath the surface of the water—from 1 to 2 feet—and far enough from the sides and bottom of the channel or reservoir in which the rating is made and from all obstructions to be beyond their influence. In addition, the meter is usually held quite rigidly in a horizontal position, on either a rod or a cable. In field use, on the contrary, the meter is used from the surface to the bottom and close to the sides of the channels throughout the entire range of depths found in the streams measured; and in high velocities there is a strong tendency for the meter to be forced out of its horizontal position. The tests discussed here were made for the purpose of determining whether the standard ratings, made as described, hold good when the meter is held near the surface of the water or near the sides or bottom of the channel or out of the horizontal. Further tests were made to determine the effect of the movement of the meter in the water when the "integration" method is used and also the effect of dulling the pivot bearing of the meter.

On September 5 and 6, 1913, a series of runs was made at the meter rating station of the California Development Co., at Calexico, Cal.[1]

[1] For a detailed description of this station, *see* Allison, J. C., Selling water by current meter measurement, *in* Engin. News, v. 69, no. 2, 1913.



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This station consists of a car, measuring accessories, and a concrete trough with a level bottom. The trough is 2 feet wide on the bottom and 3 feet deep, with side slopes of 1 to 1. At the time the experiments were made it was carefully cleaned of silt and débris and filled about three-fourths full with fresh ditch water carrying a rather high percentage of silt.

The experiments at Calexico consisted of runs with the meter on a rod and held horizontal at a depth of 1 foot below the surface of the water, practically reproducing the conditions under which it was rated, runs with the meter tipped upward and downward at various angles, runs with the pivot dulled, runs with the meter held close to the side walls of the trough, and runs with the meter just clearing the bottom of the trough. The results of these experiments, plotted to logarithmic scale, are shown in figure 1.

The meter used was rated at Chevy Chase Lake, near Washington, D. C., during the first half of May, 1913, by the United States Bureau of Standards and had been in use throughout the summer. The points developed by this original rating for the runs made with the meter equipped with a single-point contact head, as it was during the experiments at Calexico, are also shown in figure 1, for comparison with the Calexico results. The curve shown on the figure represents the results of the original rating.

For the meter submerged 1 foot, the rod held vertical, and with the pivot in good condition the points are as close to the curve of the original rating as a majority of the points in the original rating, but are all on one side of the curve, indicating that the meter was slightly faster after several months' use than at first, but the difference is so slight as to be negligible.

The runs made with the meter tipped out of the horizontal position indicate that the meter does not run true to the standard rating curve. Not enough runs were made under these conditions to develop curves, but the results indicate clearly that it is very important to keep the meter horizontal.

The runs with the meter held close to the sloping sides of the trough gave points on both sides of the standard rating curve, but indicate that the meter runs true to the rating curve under these conditions. This is shown also by experiments at Cornell University, which will be discussed later.

The runs with the meter just clearing the bottom of the concrete channel show that a correction is necessary when the meter is so used in practice. When plotted on a natural scale, the data shown in figure 1 indicate that, with the meter used, the water would actually flow about 0.06 foot per second faster than indicated by the velocity of the meter. On a natural scale the curve for the meter held at the bottom was quite parallel to the standard curve and 0.06 foot per second slower.

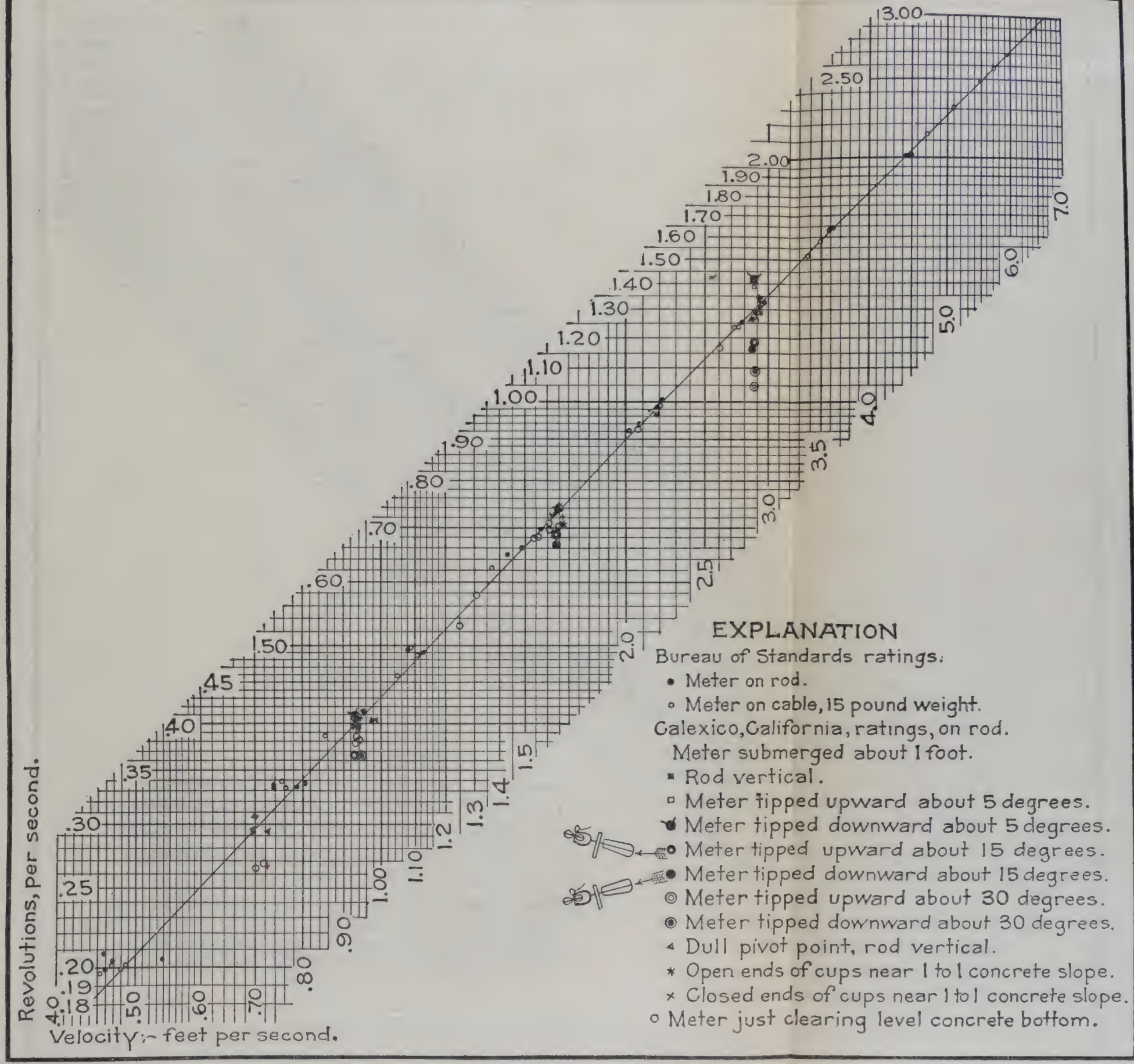



FIG. 1.—Standard-rating curve on logarithmic scale, as developed by U. S. Bureau of Standards, for new cup meter, with points developed by rating the same meter at Calexico, Cal., under conditions similar to those under which it was originally rated and under other conditions not met in the usual rating.

41217°—14. (To face page 78.)

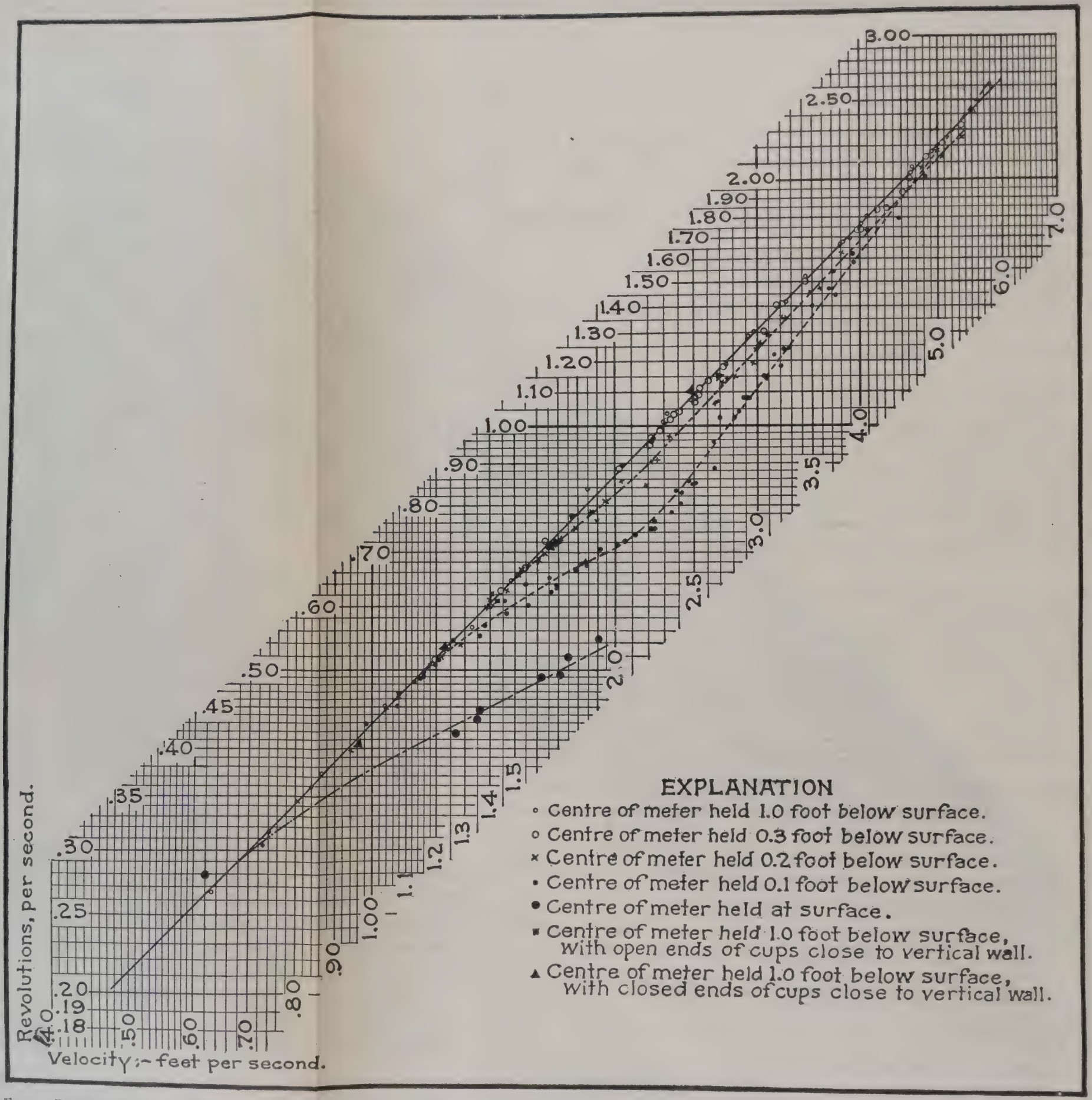



FIG. 2.—Rating curves and individual points on logarithmic scale, developed in the rating of a new cup meter at the hydraulic laboratory of Cornell University by holding the meter nearer the surface of the water and nearer the sides of the channel than is done in the usual rating.

41217°—14. (To face page 79.)

For the experiments made with a dulled pivot the pivot was roughly rounded on a carborundum wheel to a curve of about the same radius as the shaft—far duller than probably would ever be used in actual practice, as the wheel developed facets. The experiments with the very dull pivot show that for velocities of water greater than 1 foot per second the sharpness of the pivot is immaterial, the points determined by the experiments being closer to the standard curve than most of those from which this curve was developed. As might be expected, the added friction due to the rounding of the pivot has more influence in low velocities, and the meter is a little slow under these conditions.[1]

On November 29 and December 1, 1913, the writer made experiments in the concrete channel of the hydraulic laboratory of Cornell University, with a new meter of the same type as that used in the canal measurements during the summer and in the Calexico experiments. The results of these runs are plotted to logarithmic scale in figure 2.

These experiments indicate that for depths below the surface of 0.3 foot or greater the meter runs true to the standard-rating curve at least within the range of velocities covered, which did not exceed 6 feet per second. When the center of the meter is less than 0.3 foot from the surface, the points indicate a peculiar behavior. With the center 0.2 foot below the surface the meter velocities followed the standard curve until a velocity of about 1.5 feet per second was reached. At this velocity the surface of the water above the meter starts to break up and the rating curve leaves the standard curve, as shown by the dash-and-dot line in figure 2, but it did not intersect the standard curve again within the range of the experiments.

With the center of the meter submerged just 0.1 foot the curve leaves the standard curve at a lower velocity—about 1.15 feet per second—as is to be expected. The influence appears to reach a maximum at a velocity of about 2.2 feet per second, at which point the actual water velocity is about 0.45 foot per second higher than the revolutions of the meter would indicate on the basis of the standard curve. As the velocity increases, the two curves come closer together until the curve for the 0.1 foot depth appears to cross the standard curve at a velocity of about 5.5 feet per second. The writer can account for this strange action only on the theory that by the time this velocity is reached the entire surface of the water in the vicinity of the meter has become shattered, and enough wind pressure operates against the cups to turn the meter even faster than would be the case when submerged 1 foot. If a cup meter is held in the air, the great influence of a very slight wind is at once manifest.

[1] In correspondence, Mr. V. M. Cone, Irrigation Engineer, Office of Experiment Stations, points out "that the ratings did not change materially on those pointed bearings if they were merely dulled, but the big trouble came when using the meter in comparatively swift water. There was a tendency for the turbine to lift, throwing the conical bearing upward, and very often a shoulder would be cut on the sloping face. Whether this is due to a mechanical defect in the instrument or to grit getting into the bearing I do not know, but wherever that groove or shoulder was cut the rating was very materially changed."

Merely to make the data complete rather than to duplicate conditions of field use, the meter was again raised in the water until the center plane of the cups was just at the water surface. The curve for this condition shows that the meter runs slow when a low velocity is reached, and the difference between the curves rapidly becomes greater within the range of the experiments.

Murphy, in the report of his experiments at Cornell University,[1] also pointed out the fact that the Price meter does not run true to standard rating curve when held near the surface. He compared the velocity as indicated by the meter with that of a surface float operated simultaneously in running water. His general deduction was that this type of meter does not run true to standard curve when operated at depths less than 0.5 foot below the surface. The writer found, however, by actu-

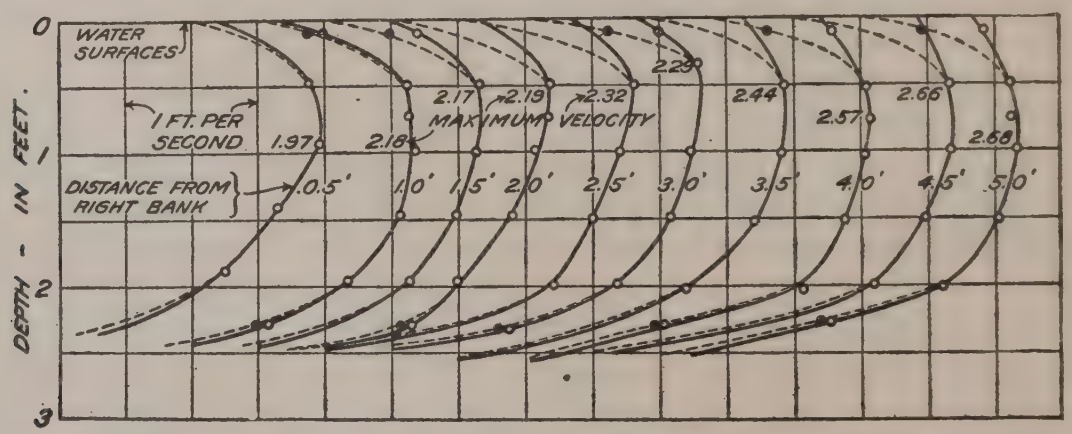



FIG. 3.—Vertical velocity curves, showing error due to using standard rating for points near the surface and bottom, Orr ditch, Nevada.

ally rating the meter at various depths, that the behavior of the meter becomes normal somewhere between depths of 0.2 and 0.3 foot below the surface.

A concrete example of the difference in results obtained when the proper correction for surface and bottom velocities is applied follows:

The Orr ditch at Reno, Nev., was measured with a current meter, using the vertical velocity-curve method. The ditch channel was lined on the sides with rubble masonry. This channel was 10 feet wide and of nearly rectangular section. The vertical velocity curves for the right half of the section are shown in figure 3. The open circles and solid lines give the curves after the proper corrections have been applied to the velocities for the surface and the bottom. The dots and broken lines show the form the curves would have assumed if no corrections had been applied. The curves for the other half of the section were quite similar to these. The verticals were spaced 0.5 foot apart. The total discharge indicated by the solid curves is 45.80 second feet, while that

[1] Murphy, E. C. Accuracy of stream measurements. U. S. Geol. Survey, Water-Supply and Irrig. Paper No. 95, 1904, p. 90.

indicated by the original curves, which followed the dotted lines of figure 3, is 44.82 second feet, which is 2.14 per cent less than the discharge computed with proper corrections applied. The mean velocity indicated by the solid curves is 1.76 feet per second. As influenced by the broken portions, it is 1.72 feet per second. For a given depth and a given mean velocity the difference in results with and without the proper corrections will vary approximately as the width of the channel, but for a given

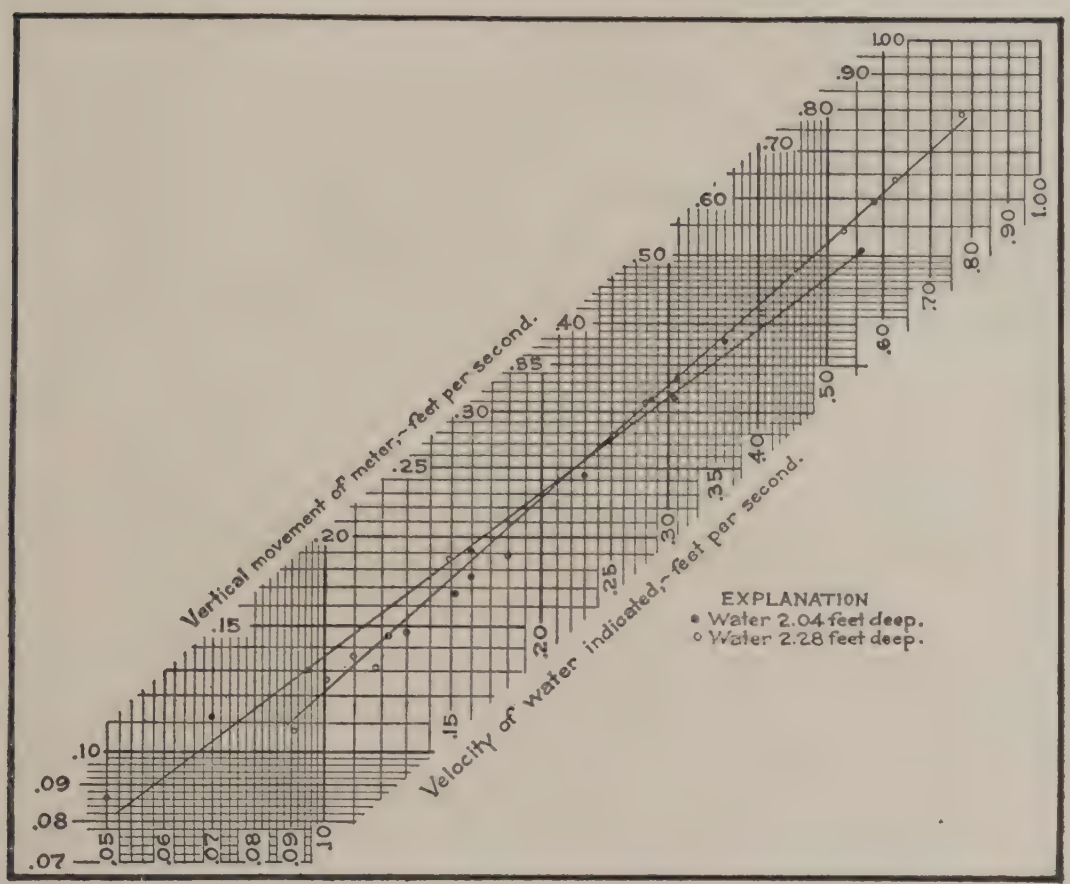



FIG. 4.—Curves on logarithmic scale, showing relation between rate of vertical movement of a cup meter and velocity of water as indicated by the revolutions of the turbine due to vertical movement in still water.

width of channel the difference approximates a constant quantity without regard to depths and velocities rather than a percentage difference.

The main point the writer desires to bring out is that a cup current meter should be actually rated at the surface and bottom of the channel if it is to be operated at these points in making discharge measurements. From the various rating tables thus developed the proper interpretation of the speed of the meter can be recorded.

Runs with the meter held close against the vertical concrete wall of the channel indicate that the meter runs true to the standard curve, but disregards the cycloidal motion of the water. As the rating car passed to and fro over the channel, the meter was held close to the same wall, thus bringing the open and closed sides [1] of the turbine, alternately, next to the wall.

For the purpose of determining the influence on a meter of the vertical motion in the water when the vertical integration method is employed, experiments were made in Lake Tahoe, Cal., on July 20, 1913. During the first 11 runs the meter was operated by the writer's assistant. The surface of the lake was smooth and the water exceptionally clear, so that the meter could be seen plainly. The particular cup which passed under the yoke at the moment of the electrical click was marked, and a definite number of round trips were made from the top of a flat rock 2.04 feet below the surface to the surface and return. By watching the marked cup it was possible to determine the fractional parts of a revolution to within probably one-fifth of a revolution. A slight breeze sprang up later in the day, and the meter was taken to a protected place where the water was clear, 2.28 feet deep, and without a ripple. For the runs made in this depth of water the writer operated the meter.

In order to give a clearer understanding of the plotted results shown in figure 4, information in detail of the separate runs is given in Table I.

TABLE I.—*Indicated velocity of water due to vertical movement of cup meter in still waters of Lake Tahoe, Cal.*

| Run No. | Depth of water. | Number of round trips. | Time. | Number of revolutions. | Vertical movement of meter per second.[1] | Number of revolutions per second. | Indicated velocity of water per second. |
|---|---|---|---|---|---|---|---|
| | *Feet.* | | *Seconds.* | | *Feet.* | | *Feet.* |
| 1 | 2.04 | 1 | 69.4 | 0.0 | 0.053 | 0.000 | 0.000 |
| 2 | 2.04 | 4 | 77.0 | 4.5 | .191 | .058 | .160 |
| 3 | 2.04 | 4 | 60.4 | 5.5 | .244 | .091 | .230 |
| 4 | 2.04 | 4 | 83.8 | 5.0 | .176 | .060 | .160 |
| 5 | 2.04 | 3 | 84.8 | 2.3 | .130 | .027 | .095 |
| 6 | 2.04 | 3 | 98.8 | 1.7 | .112 | .017 | .070 |
| 7 | 2.04 | 3 | 128.6 | 1.2 | .086 | .009 | .050 |
| 8 | 2.04 | 5 | 48.8 | 7.2 | .377 | .148 | .360 |
| 9 | 2.04 | 5 | 36.4 | 8.7 | .506 | .239 | .560 |
| 10 | 2.04 | 9 | 105.4 | 13.2 | .314 | .125 | .307 |
| 11 | 2.04 | 2 | 44.2 | 2.4 | .167 | .054 | .152 |
| 12 | 2.28 | 3 | 57.2 | 3.8 | .218 | .067 | .180 |
| 13 | 2.28 | 3 | 40.4 | 4.5 | .309 | .111 | .280 |
| 14 | 2.28 | 3 | 40.2 | 4.6 | .311 | .114 | .285 |
| 15 | 2.28 | 3 | 67.2 | 3.6 | .186 | .054 | .149 |
| 16 | 2.28 | 2 | 61.2 | 2.1 | .136 | .034 | .110 |
| 17 | 2.28 | 2 | 56.6 | 2.5 | .147 | .044 | .130 |
| 18 | 2.28 | 2 | 65.8 | 2.1 | .126 | .032 | .101 |
| 19 | 2.28 | 5 | 50.0 | 8.5 | .416 | .170 | .408 |
| 20 | 2.28 | 7 | 49.0 | 12.3 | .594 | .251 | .585 |
| 21 | 2.28 | 5 | 38.5 | 8.7 | .540 | .226 | .530 |
| 22 | 2.28 | 7 | 37.0 | 12.5 | .788 | .338 | .778 |
| 23 | 2.28 | 8 | 52.2 | 14.0 | .637 | .270 | .625 |
| 24 | 2.28 | 2 | 63.4 | 2.5 | .131 | .039 | .118 |
| 25 | 2.28 | 2 | 77.8 | 2.2 | .107 | .028 | .091 |
| 26 | 2.28 | 3 | 37.4 | 4.7 | .334 | .126 | .310 |
| 27 | 2.28 | 3 | 86.2 | 3.7 | .145 | .043 | .123 |
| 28 | 2.28 | 4 | 88.6 | 6.0 | .188 | .068 | .180 |

[1] For one round trip the meter moves 0.4 foot less than twice the measured depth of water.

The curves plotted to logarithmic scale are based on points whose ordinates represent the velocity in feet per second at which the meter was drawn through the water (column 6), and the abscissas represent the velocity of water indicated by the number of revolutions generated

[1] By "open side of turbine" is meant the side on which the open ends of the cups are upstream

by the vertical movement on the basis of the standard rating (column 8). The two curves show a slight difference, probably due to two causes: First, the difference in depth of the water and the consequent difference in the relation between the number of turns at top and bottom and the distance through which the meter travels. Second, a difference in the manner in which different men operate the meter. For the higher rates of movement of the meter the points for the curve appear quite consistent. This is due to the fact that the turbine rotates all the time, even in still water, when the vertical movement is faster than about 0.2 foot per second. Below that velocity of movement the turbine rotates part of the time and remains at rest part of the time. It was noticed that the turbine rotates part of a revolution when the motion was changed from up to down, and vice versa. At these points the turbine rotated a little and then came to rest again and remained so while the meter was being moved in a vertical direction to the next turning point.

A general deduction from these curves is to the effect that all vertical movement of the meter tends to turn the wheel in the same direction as does the flowing water. For the slower movements of the meter this tendency is not sufficient to overcome the internal friction in the meter, but, when it is operated in running water, the water already has overcome this internal friction, and the force due to the vertical movement of the water is still available to turn the wheel and thus cause it to overregister.

Again referring to figures 1 and 2, it appears that a cup meter underregisters at all points nearer the surface than about 0.3 foot and also while very near the bottom. Therefore, in a process of vertical integration with a cup meter there is a tendency to overregistration at all times due to the vertical motion, and a tendency to underregistration while the meter is passing from about 0.3 foot below the surface up to the surface and back to that point and while near the bottom. These compensating tendencies account for the fact that if the meter is moved very uniformly and very slowly the integration method gives results as close to actual discharge as it does.

# EREMOCITRUS, A NEW GENUS OF HARDY, DROUTH-RESISTANT CITROUS FRUITS FROM AUSTRALIA

By WALTER T. SWINGLE,
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A survey of the plants belonging to the orange subfamily (Citratae), undertaken in the hope of finding new material for use in breeding, has brought to light a citrous plant which combines in some degree the winter dormancy of the kumquat and the absolute cold resistance of the trifoliate orange. This is the Australian desert kumquat, originally described as *Triphasia glauca* Lindl. and now called *Atalantia glauca* (Lindl.) Benth. by most botanists.

A study of the reports of the early Australian exploring expeditions has shown that this plant is undoubtedly the most cold-resistant of all the evergreen citrous fruits, that it is also drouth-resistant, and yields an edible, though small, kumquat-like fruit. With these qualities it combines a high degree of winter dormancy.

An examination of the scanty material of this plant preserved in the herbaria of Europe and America and a study of living plants now growing in the greenhouses of the Department of Agriculture at Washington, D. C., show that its relationships have been misunderstood by botanists. When first described it was wrongly put into the genus Triphasia and is still out of place in the genus Atalantia, to which it is now referred. As a matter of fact, the Australian desert kumquat is much more closely related to Citrus than are either of the genera just named. It is most closely related to the Australian species of Citrus and, like them, can in all probability be hybridized with the commonly cultivated species of Citrus. It can be grafted readily on the common citrous stocks.

The Australian desert kumquat is in many ways unique. It is the only member of the subfamily Citratae that shows marked adaptation to desert climates. It is a gray-green shrub or small tree, looking not unlike a large, thorny sage bush, having leaves centric in structure, with appressed hairs, stomates, and a very thick-walled epidermis on both surfaces and palisade tissue just beneath. The slender, usually spiny twigs (fig. 1) are also gray-green and have stomates situated at the bottom of deep pits. In all of these and in some other characters the plant shows the outward signs of a profound adaptation to withstand the extreme heat and dryness of a desert climate.

Because of these unique structural peculiarities and because of decided differences in the number, arrangement, and character of the floral

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organs, it seems necessary to create a new genus to include this interesting plant.[1]

**Eremocitrus** Swingle.

The genus Eremocitrus resembles Citrus in the structure and appearance of the fruits; it differs from it (1) in the leaves which have on both surfaces palisade cells, sunken stomates, and appressed few-celled hairs; (2) in the 4- to 5-merous flowers, with free stamens and a 4- or 5-celled ovary, with 2 ovules in each cell.

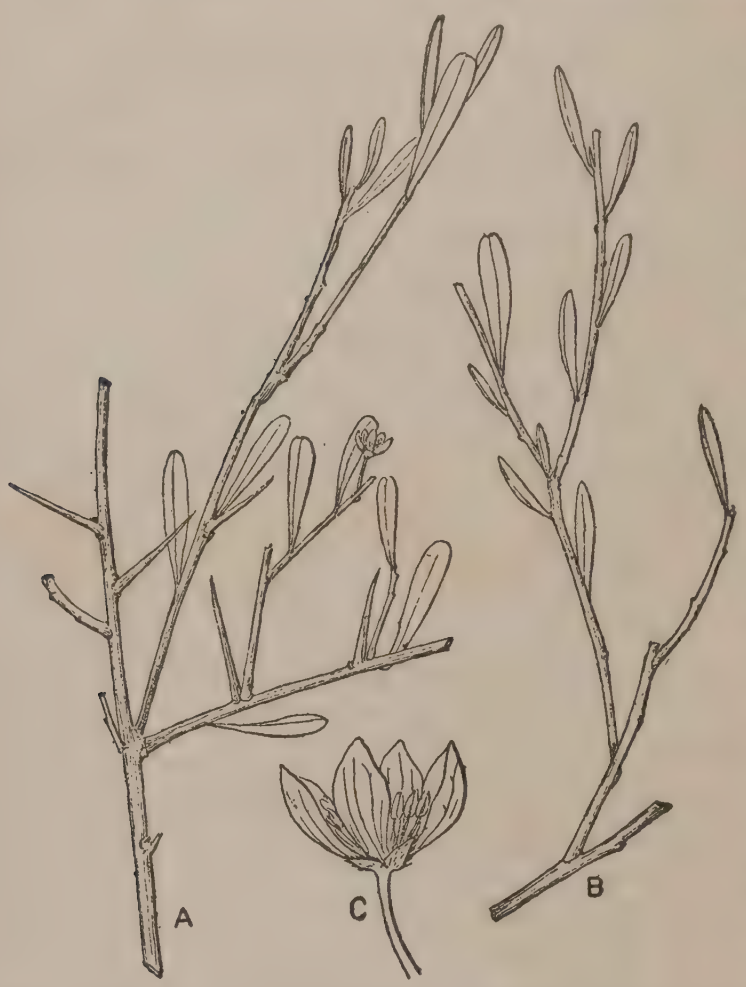



FIG. 1.—*Eremocitrus glauca:* Twigs from a syntypic specimen collected by T. L. Mitchell, near Forestvale, Queensland, Australia, on October 17, 1846; from Gray Herbarium. *A*, Spiny twig showing a single flower; *B*, spineless sterile twig; natural size. *C*, tetramerous flower seen from the side. ×5. Drawn by Theodor Holm.

The leaves are gray-green, thick and leathery, and markedly pellucid punctate; they are nearly alike on both sides, having four ventral and two dorsal layers of palisade cells, sunken stomates, an epidermis with a thick cuticle, and scattered few-celled appressed hairs on both surfaces. The spines are usually long and slender, but are sometimes wanting, especially on fruiting branches of old trees. They occur singly in the axils of the leaves. The twigs are gray-green, slender, very slightly angled when young, with scattered stomates at the base of deep, narrow pits, and two or more layers of palisade cells below the very thick-walled epidermis. The flowers occur singly or two or three together in the axils of the leaves and are borne on slender pedicels about as long or slightly longer than the petals. (See fig. 1.) The calyx is 3- to 5-lobed; the petals, four or five, rarely three in number, are more or less narrowed at the base;

[1] EREMOCITRUS gen. nov. (ἔρημος deserta + Citrus).—Genus *Citro* affinis, foliis utrinque stomatibus pilisque instructis; floribus 4- vel 5-meris, staminibus liberis, ovario 4- vel 5-loculare, loculis 2-spermis.

Folia cinereo-viridia, crassiuscula, pellucido-punctata, mesophyllo centrico, utrinque stomatibus et pilis adpressis, pauci-cellularibus instructa. Spinae in axillis foliorum singulae, interdum in ramis veteris carentes. Flores in axillis foliorum singulae vel paucae, pedicellatae, 4- vel 5-meres; petala basi angustata; stamina libera, numero petalorum quadruplo; ovarium 4- vel 5-loculare, ovulis in locula binis; stylus brevi-usculus, crassus, caducus. Fructus ovalis vel pyriformis, cortice ut in *Citro* carnosa, glandulis oleiferis instructa; pulpa vesiculari acida, vesiculis subglobosis pedicellatis. Semina parva, 5 mm. longa; testa dura rugosa; cotyledones hypogaeae; folia primitiva cataphylla.

Arbuscula vel arbor parva, spinosa.

Species typica et unica, *Triphasia glauca* Lindl.

the stamens are normally four times as numerous as the petals, with the filaments free. The ovary is obovate, with a rather thick, subcylindric, caducous style, 4- or 5-celled, with two ovules in each cell; the disk is small. The fruits are 1½ to 2½ by 1¼ to 1½ cm.—smaller than those of any known species of Citrus—subglobose, oval or somewhat pyriform, with a thin, fleshy peel, like that of a lime, covered with oil glands. The pulp is vesicular, sour, and juicy. The pulp vesicles, which separate easily in the ripe fruit (fig. 2, *A–D*), are subglobose, and are borne on slender stalks. The seeds are small (5 to 6 by 3 to 4 by 2½ to 3 mm.), pointed ovate, yellowish gray with a hard testa, irregularly verrucose and furrowed in a longitudinal direction. (See fig. 2, *E*.) The cotyledons are plano-convex, remaining hypogeous in germination; the postcotyledonary leaves are slender cataphylls (fig. 3).

This monotypic genus is based on *Triphasia glauca* Lindl., native to the drier parts of northeastern Australia.

## RELATIONSHIPS

Eremocitrus is most nearly related to the three aberrant Australian species of Citrus, *C. australis* Planch., *C. australasica* F. Muell., and *C. Garrowayi* F. M. Bail., and agrees with them in having free stamens, subglobose pulp vesicles, and the first postcotyledonary leaves of the young seedlings reduced to cataphylls. It differs from these Australian species of Citrus in having gray-green leaves, with palisade cells, stomates, and curious appressed hairs on both surfaces, and also in having the stomates of the twigs situated at the base of pits, in the usually 4-merous flowers, and in the much smaller fruits with only one or two seeds in a segment.

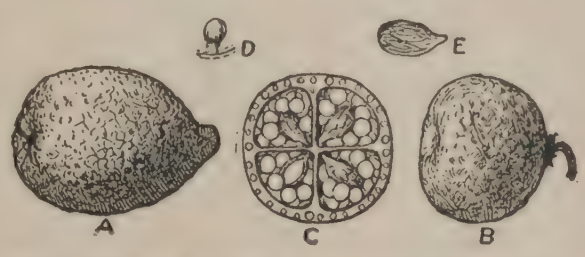



FIG. 2.—*Eremocitrus glauca:* Fruits and seed from Tambo, Queensland, Australia (S. P. I. No. 29537, James Pink, January, 1911). *A*, large pyriform fruit, natural size; *B*, small oblate fruit, natural size; *C*, cross section of a 4-celled fruit, showing four seeds and numerous pulp vesicles, natural size; *D*, a single, short-stalked pulp vesicle. ×2; *E*, seed, showing rugose testa. ×1⅓. Drawn by M. W. Gill.

Eremocitrus resembles the kumquat (*Citrus japonica* Thunb.) in being a shrub bearing very small fruits and in its physiological adaptations to secure extreme winter dormancy. The kumquats differ decidedly from it in having usually 5-merous flowers, polyadelphous stamens, and a 5- or 6-celled ovary, short-stalked, fusiform pulp vesicles, and bifacial glabrous leaves. Eremocitrus shows little affinity to any other species of Citrus.

From true Atalantias, such as *A. monophylla* (Roxb.) DC. and *A. citrioides* Pierre, having 2- to 4-celled fruits with pulp vesicles, Eremocitrus differs in having the stamens four times as numerous as the petals instead of twice as numerous. It also differs markedly from Atalantia in the structure of the leaves. It differs from the African cherry oranges (*Citropsis* spp.) in having simple leaves, two ovules in each cell of the ovary, stalked pulp vesicles, and in many anatomical characters.

In its fundamental morphological characters, Eremocitrus probably represents fairly accurately the ancestral type from which were derived the Australian species of Citrus. This ancestral type has been, however, profoundly modified by the superposition of many anatomical and physiological characters acquired as a result of a long-continued struggle for life in a desert climate.

Only one species of Eremocitrus is known:

**Eremocitrus glauca** (Lindl.), n. comb.

*Triphasia glauca* Lindl., *ex* T. L. Mitch., 1848. Jour. Exped. Trop. Austral. p. 353. London.

*Atalantia glauca* Benth., 1863, Fl. Austral., v. 1, p. 370. London.

Illus., W. S. Campbell, 1899, *in* Agr. Gaz., N. S. Wales, v. 10, p. 1168, fig. 5, sub. nomen *Citrus australis* (sterile twigs); Fairchild, 1912, *in* U. S. Dept. Agr. Yearbook, 1911, pl. 45, fig. 1 (fruits only).

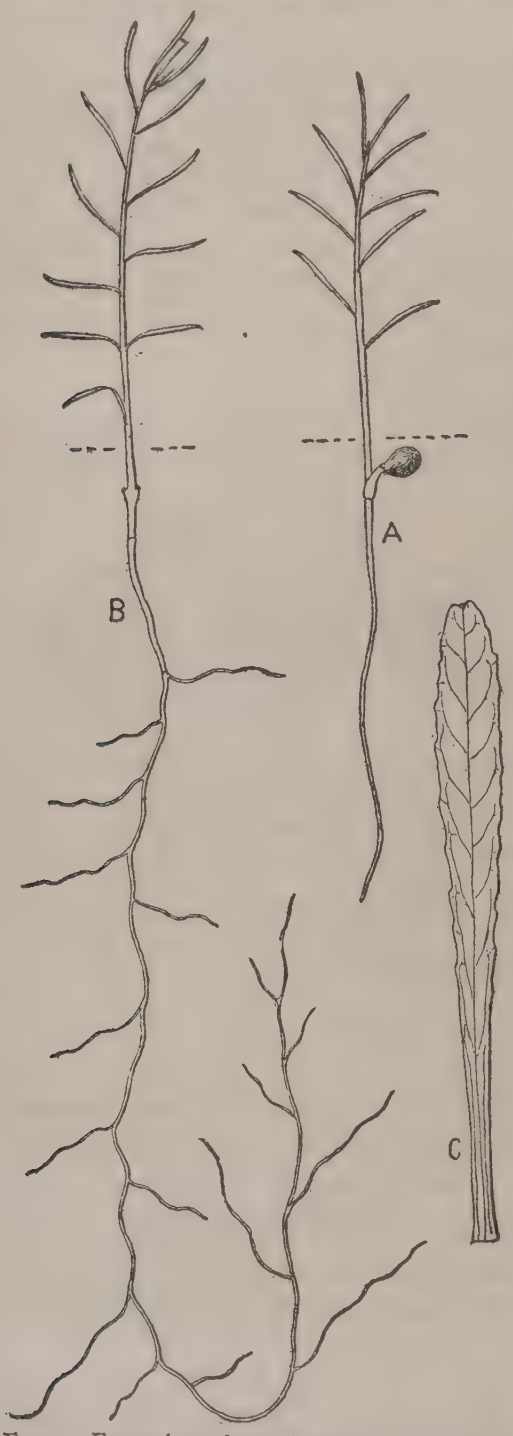



FIG. 3.—*Eremocitrus glauca:* Seedling plants grown from seed from near Chinchilla, Queensland, Australia (S. P. I. No. 29660). *A*, young seedling with hypogeous cotyledons still inclosed in the seed coats, natural size; *B*, an older seedling with a very long taproot, natural size; *C*, a cataphyll. ×5. Drawn by Theodor Holm.

This species, the desert kumquat, desert lemon, or desert lime of the Australian pioneers, is a shrub or small tree sometimes attaining a height of 15 feet and a diameter of 6 inches (Maiden, 1889, p. 379).[1] When young, the branches are very spiny and the leaves are very narrow. As the tree gets older, the leaves become broader and more abundant and the spines are much reduced or entirely wanting (Campbell, 1899, p. 1168, fig. 5).

The leaves of mature plants are oblong linear or elongate cuneate, bluntly rounded, retuse or emarginate at the tip, with undulate entire margins, 25 to 45 by 4 to 10 mm., mostly 30 to 40 by 6 to 8 mm. They show on both surfaces minute (about $\frac{1}{10}$ mm. long), scattered, appressed few-celled hairs, with a warty cuticle. The leaves are paraheliotropic (standing more or less on edge), very thick, prominently glandular dotted, and taper gradually into very short wingless petioles.

[1] Bibliographic citations in parentheses refer to "Literature cited," pp. 99–100.

The spines, which are always single, are slightly to one side of the axil of the leaf and are usually very slender, 2 to 4 cm. long and only 1½ to 2 mm. in diameter. On old trees, especially on fruiting branches, they are often wanting.

The flowers are borne either singly or in groups of two or three in the axils of the leaves on new growth, as in Citrus. The pedicels are slender, 4 to 6 mm. long. The calyx is 3- to 5-lobed, sparsely hairy, the lobes acute. The petals are four or five (rarely three) in number, somewhat narrowed at the base, and broadly rounded or bluntly pointed at the tip, 4 to 6 mm. long. There are four times as many stamens as petals, usually 16 to 20, rarely 12 (in trimerous flowers); the filaments are slender, about 4 to 5 mm. long. The pistil is borne on a low disk and has an obovate ovary, with a rather thick subcylindric style (fig. 4); the ovary is 4- or rarely 3- to 5-celled; each cell contains two ovules.



FIG. 4.—*Eremocitrus glauca:* Flower from which petals and stamens have fallen; showing pedicel, calyx (one sepal cut away), disk, ovary, style, and stigma; from a specimen collected by Robert Brown, No. 58, Sept., 1802 (?), Upper Head, Broad Sound, Queensland; in National Herbarium, Washington, D. C. ×5. Drawn by J. M. Shull.

The fruits are small, globose, oblate, or sometimes pyriform, 1½ to 2½ by 1¼ to 1½ cm., having four (rarely three or five) cells filled with subglobose stalked pulp vesicles. The seeds are oval, yellowish gray, 5 to 6 by 3 to 4 by 2½ to 3 mm., with a tough, longitudinally furrowed, and verrucose testa (see fig. 2). The cotyledons are hypogeous in germination, and the young seedlings produce alternate slender cataphylls which only very gradually become broader and leaf-like. The young spiny plants, even when several years old, usually have only very narrow leaves, differing but slightly from the cataphylls of the young seedlings. (See fig. 3.)

## DISTRIBUTION OF EREMOCITRUS

Eremocitrus occurs in northeastern Australia from the Burdekin River, Queensland, latitude 21° S. (Mueller, 1858, p. 150; 1857, p. 169; Gregory, 1857, p. 237), to Dubbo, New South Wales, latitude 32° 30′ S.; also in the coast region near Broad Sound in Queensland, latitude 22° S. Specimens have been examined from the following localities:[1]

I. QUEENSLAND.

PORT CURTIS DISTRICT.—**Broad Sound.** ROBERT BROWN, No. 5343, September, 1802 [?] "Rutac ? suaveolens Aurantiac. genus 10 and. bacc. polysperma No. 58 desc. nost. [?] a Broad Sound. Ad margines dumetis prope Upper Head, Broad Sound." Three twigs, two with flowers, one with young fruits, British Museum; fragment with flowers, Washington, D. C. (National Herbarium); no number, no date, four twigs, two with flowers, two with young fruits, Kew;[2] fragment in Washington, D. C.

[1] All of the specimens located in herbaria outside of Washington, D. C., were examined and photographed by the writer, and prints enlarged to natural size have been filed in the National Herbarium at Washington, D. C.

[2] This specimen has a locality label "Broad Sound" in Robert Brown's handwriting, but no other data. It is probably a part of No. 58, as the specimens are similar to those in the British Museum.

(National Herbarium); no number, no date, two sheets of two and three sterile twigs, Paris (Muséum) [1]; no number, no date, four sterile twigs, Dahlem.[1]

North Kennedy District.—Brigalow Scrub, south of **Burdekin River,** between latitude 20° and 21° S. Ferdinand Mueller, no number, October 14 to 30, 1856 (Mueller, 1857, p. 169, 1858, p. 150); three twigs, one with flower and two with fruits, Kew.

South Kennedy District.—Head of **Suttor River.** Rutherland, no number, no date [before 1867], two twigs, one with young fruits, one sterile, Kew.

Maranoa District.—Dublin County, near **Forestvale,** between Possession Creek and "Camp 32," near the junction of Maranoa and Merevale Rivers. T. L. Mitchell, October 17, 1846, three sheets with flowering twigs,[2] Kew; "Interior of New South Wales, Sir T. L. Mitchell, Presented 1847," two twigs, one with fruits, British Museum; two twigs, one with flower (see fig. 1), Harvard (Gray Herbarium).

Warrego District.—F. M. Bailey, no number, no date (before 1911), two twigs, one with flower buds, one sterile, Washington, D. C. (National Herbarium).

In vicinity of **Tambo.** Collected for James Pink (S. P. I. No. 29537), January, 1911; fruits only (fig. 2; see also Pl. XLV, fig. 1, U. S. Dept. of Agr. Yearbook for 1911), Washington, D. C. (Department of Agriculture Economic Collections, National Herbarium); Walter T. Swingle (C. P. B. No. 7239), February 20, 1914; twigs cut from greenhouse plants grown from seed sent by James Pink, Washington, D. C. (National Herbarium).

Darling Downs District.—Lytton County, near **Chinchilla.** "Growing naturally on the Condamine River, 12 miles from Chinchilla Rly. Station," collected for John Williams (S. P. I. No. 29660), December, 1910, seeds only, Washington, D. C. (Department of Agriculture Economic Collections, National Herbarium); Walter T. Swingle (C. P. B. No. 7244), February 20, 1914, leafy twigs from plants grown in Department of Agriculture greenhouse from seed sent by John Williams, Washington, D. C. (National Herbarium).

Aubigny County, **Dalby.** T. L. Bancroft, no number, no date, "Desert or native kumquat, used by settlers," one twig with flowers, Washington, D. C. (National Herbarium).

Marsh County, **Goondiwindi.** Collected for J. H. Maiden, no number, May, 1912, twig and ripe fruits, Washington, D. C. (National Herbarium); Walter T. Swingle (C. P. B. No. 7522), February 20, 1914, leafy twigs from plant grown in Department of Agriculture greenhouse from seed sent by J. H. Maiden, Washington, D. C. (National Herbarium).

II. New South Wales.

Lincoln County, **Dubbo.** Collected for J. H. Maiden (C. P. B. No. 2901), April 22, 1910, fruits only, Washington, D. C. (National Herbarium).

Finch County, **Collarenebri.** Collected for J. H. Maiden (S. P. I. No. 37808) before March 16, 1914; twigs, leaves, and fruits, Washington, D. C. (National Herbarium).

[1] This material has no original label, but is probably from the same collection as No. 58. All of these specimens show pedicels which have lost the flowers or young fruits.

[2] *Triphasia glauca* was probably based by Lindley on several syntypes, and it is now difficult to decide which specimen should be designated as the lectotype. One sheet (Herb. Hook., 1867) with four twigs has pencil sketches of the flower parts by "J. F." on a separate sheet. One with two twigs (Herb. Benth., 1854) has a printed label like that on the specimen in Gray Herbarium. The third specimen (Herb. Hook., 1867), also with two twigs, has pencil sketches on the sheet of flower parts and the following notes: "Cal. lobes 4–5, small, unequal imbricate ciliate. Petals 4–5 fleshy, unequal, concave, sessile[?]. Stamens 15–20, anthers oblong, sessile[?], 2 loc. 2 longit. rim. Ovary." Above these notes the names "Dodonaea," "Celastrus," and "Cfr Diosma" are written as if in an attempt to identify the plant. On the same sheet are mounted three twigs collected by Mueller on the Burdekin River. These eight twigs in the Kew Herbarium, together with the two in the British Museum and the two in the Gray Herbarium, are probably syntypes.

The first specimens of *Eremocitrus glauca* to be collected in Australia were found by Robert Brown in September, 1802(?), in the thorny brush at Upper Head on the coast region bordering Broad Sound in Queensland and not in its characteristic habitat, the Downs or Brigalow Scrub country, to the west of the coast ranges in southern Queensland and New South Wales. Brown's specimens, the best of which are now found in the British Museum, show unusually abundant leaves and flowers, probably because they were growing in a somewhat moister climate than usual. Either this colony of the desert kumquat represents a slightly different subspecies, or else it is merely a chance colony of the typical form of the species introduced into the coast region by birds or by other means. It differs only in its somewhat luxuriant growth from the desert kumquat of the semiarid scrubs to the west of the coast ranges.

## HARDINESS OF THE DESERT KUMQUAT

The accounts of *Eremocitrus glauca* contained in the records of the early exploring expeditions in Australia show it to be native to the semiarid region west of the coastal mountains of Queensland and New South Wales, where drouths are frequent and the temperature often falls low in winter. These records are of interest, as they reveal that this near relative of our citrous fruits has great resistance to cold and drouth.

The first mention as yet found of this plant is in Ludwig Leichhardt's narrative of his expedition from near Brisbane to the Gulf of Carpentaria made in 1844–45 (1847, p. 8, 43, 75, 83, and map). During the months of November and December, 1844, Leichhardt and his party traveled from the Darling Downs, at the headwaters of the Condamine River, about 100 miles northwest of Brisbane, to the Expedition Range, some 300 miles to the northwest. This route was in general parallel to the coast, about 100 to 150 miles inland, and just westward of the coast range for the first half of the distance. The Brigalow Scrub, a dense growth of scrubs and small trees, taking its name from the Brigalow (a species of Acacia), whose silvery green leaves give a distinctive appearance to the thickets where it abounds, occupies much of this low plateau region west of the coast ranges, and generally covers the heavy loam soil that overlies the sandstone formation.

Leichhardt, during this portion of his trip, notes repeatedly the presence of a "small orange" or "native lemon." He first found this plant on October 5, 1844, while the party was camping on the Darling Downs.[1] This was in latitude 26° 56′ 11″ S., and, though the antipodean spring was well advanced (*Eremocitrus glauca* was in flower when seen here), the thermometer had registered 32° F. at sunrise two days previously.

[1] "A small orange tree, about 5–8′ high, grows either socially or scattered in the open scrub * * *." (Leichhardt, 1847, p. 8.)

On November 20, in traveling along Robinsons Creek in latitude about 25° 30′ S., he notes (Leichhardt, 1847, p. 43) that:

* * * the country began to rise into irregular scrubby ridges; the scrub generally composed of Vitex intermingled with various forest trees. The small orange-tree, which we had found in blossom at the Condamine, was setting its fruit.

The last mention of the plant was made on December 24, while the party was in the region between the Expedition and Christmas Ranges, latitude 24° 30′ to 45′ S. On that day Leichhardt (1847, p. 83) observed:

Here we passed an extensive Myal forest, the finest I had seen, covering the hilly and undulating country, interspersed with groves of the native lemon tree; a few of which were still sufficiently in fruit to afford us some refreshment.

On the night of December 14–15, when in the same country, only a few miles away, cold weather was encountered, of which Leichhardt wrote (1847, p. 75):

The night was extremely cold, notwithstanding we were encamped under the shelter of trees: and it was therefore evident that we were at a considerable elevation above the level of the sea.

The next account of this species is by Lieut. Col. Mitchell (1848, pp. 188, 209, 221, 231, 353, 434, and maps). This plant was discovered on October 17, 1846, not far from his camp, No. 32, near the juncture of the Maranoa and Merevale Rivers, in the southern part of Queensland, latitude 26° S.[1] The drouthy character of the climate is evident from Mitchell's remark (1848, p. 353) that:

We had this day passed over a fine open forest country, in which were also groves of the *Acacia pendula*. The vegetation, in general, seemed drooping, from the want of rain; but the whole was available for grazing purposes.

Among the interesting plants collected was the one in question, of which Mitchell (1848, p. 353) wrote:

A small fruit, with the fragrance of an orange, proved to be a new species of *Triphasia*.†

†*T. glauca* (Lindl. MS.); spinosa, foliis coriaceis integerrimis crenatisque linearibus glaucis obtusis retusisque, floribus trimeris dodecandris 2–3nis brevi-pedicellatis.

The footnote inserted at this point is the original botanical description of the plant.

This plant was found on the return trip to the south, but the expedition had passed through this same region on the way north during June and July (midwinter in that latitude), and the observations made at that time showed the climate to be decidedly cold for a country so near the Tropics. On June 24 Col. Mitchell stated that "the hoar frost had stiffened the grass and the water was frozen so that the horses cared not to drink." The temperature was 17° F. at 4 a. m. and 21° F. at sunrise. At this date his party was in the vicinity of Mount Owen, only about 45 miles north-northwest of camp No. 32. On July 2, at camp No. 41, in the valley of the Warrego River (latitude 25° S., longitude 147° 30″ E.)

[1] This locality is not far from the present village of Forestvale in Dublin County.

the thermometer registered as low as 7° F. at 3 o'clock in the morning and at sunrise 14° F. This was at a point 70 miles north-northwest from the region where the desert kumquat was originally found, but at 1,800 feet altitude instead of 1,300. On July 12, at his camp No. 48, at a point about 100 miles northwest of the locality where *Eremocitrus glauca* was discovered, Mitchell states that the temperature at sunrise was 11° F. This was latitude 24′ 50″ S.

On June 3 while in the vicinity of Kings Ferry,[1] some 15 miles south of camp No. 32, the type locality of the species, the temperature registered 16° F. at sunrise, which would indicate a possible minimum temperature of about 8° to 10° F., if the same values hold here as at camp No. 41. This was at an altitude of 1,400 feet, nearly the same as at camp No. 32.

It would not be surprising, in view of these scanty records taken at random, if temperatures as low as 5° F., or even zero Fahrenheit, would be found to occur occasionally in the region where the desert kumquat grows wild. Such low temperatures might injure the leaves and perhaps the smaller twigs, but recovery would probably be rapid and complete. Certainly no other edible citrous fruit is native to any region where it is exposed to such severe cold weather.

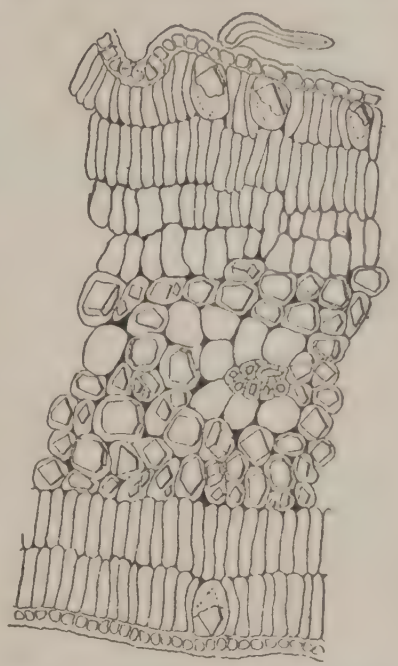


FIG. 5.—*Eremocitrus glauca:* Cross section of leaf from near Forestvale, Queensland, collected by T. L. Mitchell on October 17, 1846; in Gray Herbarium. Shows four ventral and two dorsal strata of palisade cells, central thin-walled cells often containing crystals of calcium oxalate. The outer wall of the epidermis is very thick. A stomate and an appressed hair are shown on the upper surface; these organs occur also on the lower surface. ×240. Drawn by Theodor Holm.

## DROUTH-RESISTANT ADAPTATIONS OF THE DESERT KUMQUAT

The very first glance at a specimen of *Eremocitrus glauca* shows that it is a pronounced desert plant. Any one accustomed to seeing desert plants will be struck by the familiar gray-green color of the scanty foliage of small, thick, leathery leaves. So marked is the xerophytic character of the Australian desert kumquat that an experienced botanist on first seeing it exclaimed, "It must be some kind of sagebrush!"

The cuticle is very thick, and the breathing pores are sunk below the surface and have very small air spaces below them. The leaves differ markedly from those of the ordinary citrous fruits in being provided with stomates and palisade tissue on both faces (fig. 5), making the upper

[1] This was near the present town of Donnybrook in Dublin County, Maranoa District.

and lower surfaces very similar in appearance and function, a not uncommon characteristic of desert plants. The leaves are very thick, perhaps because of their double equipment of palisade tissue. The young leaves have a scattered covering of minute short, thick appressed hairs, with a warty cuticle. They bend abruptly at right angles near the base and are from 80 to 135 $\mu$ long and 10 to 16 $\mu$ wide, often unicellular when young, but usually 2- to 4-septate when old. The wall is thickened internally at the base where it is bent, and the cuticle shows numerous wartlike, oval prominences (fig. 6). These hairs often disappear almost entirely from old leaves. Exactly similar hairs occur on the young twigs. No such persistent coating of hairs occurs on the leaves of any other citrous fruit. It is probable that a study of this plant in the field will show that this coating of peculiar hairs serves as a protection to the young twigs and leaves as they are developing.

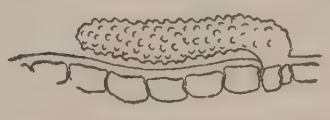


FIG. 6.—*Eremocitrus glauca:* A hair from the leaf of a plant grown from seed from near Chinchilla, Queensland (S.P.I. No. 29660). Shows abrupt bend near the base and the cuticular prominences. Such hairs are usually 2- to 4-septate. ×372. Drawn by Theodor Holm.

The young twigs of the Australian desert kumquat are very slender in comparison with those of other citrous trees and are, like the leaves, of a gray-green color. A cross section of a young twig shows the presence of a very thick cuticle and small deeply sunken stomates nearly destitute of air spaces (fig. 7). Both of these characters indicate strongly marked drouth-resistant adaptation. The twigs have below the epidermis a double layer of palisade cells filled with chlorophyll (see fig. 7) and are as well adapted to manufacture starchy food as are the leaves themselves. Here, again, we find a character common in desert plants, which often drop their leaves in time of severe drouth and utilize their green twigs for as much photosynthesis as the scanty water supply will permit.

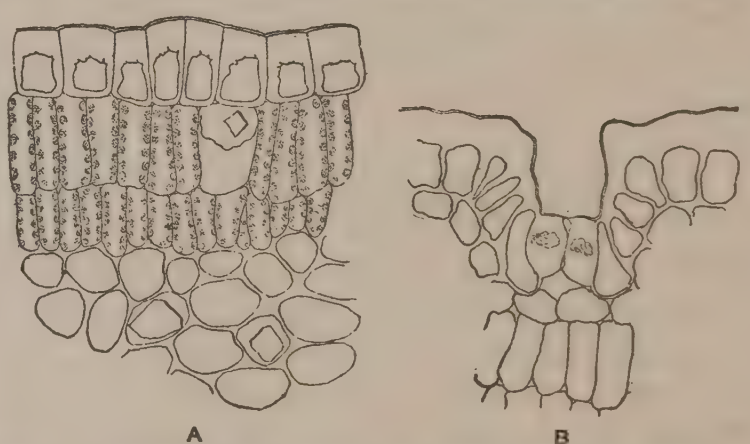



FIG. 7.—*Eremocitrus glauca:* Cross section of the epidermal region of a 1-year-old twig from near Forestvale, Queensland, collected by T. L. Mitchell on October 17, 1846; Gray Herbarium. ×248. *A*, thick-walled epidermis, two strata of palisade cells, and inner cortical parenchyma; *B*, a stomate at the bottom of a deep narrow pit. Drawn by Theodor Holm.

That this species must endure severe drouth in its Australian habitat is confirmed by the fact that on the day the type specimens were collected Mitchell observed the wilting of some of the Australian acacias, themselves drouth-resistant plants. A study of the anatomy of the desert kumquat confirms abundantly the natural inference that any

citrous fruit to thrive in such conditions must possess marked adaptations to enable it to resist severe drouth.

It is very probable that the ability of this plant to grow in dry soils exposed to hot, dry winds wlll render it of great value in breeding new types of citrous fruits better fitted than any we now possess to grow under semiarid conditions.

## USES OF THE FRUITS OF THE DESERT KUMQUAT

The early explorers of the deserts of southern Queensland and northern New South Wales note repeatedly the fact that the fruits of the desert kumquat are edible.

Leichhardt (1847, p. 77), writing on December 16, 1844, said:

> Yesterday in coming through the scrub, we had collected a large quantity of ripe native lemons, . . . we made them into a dish very like gooseberry-fool; they had a very pleasant acid taste, and were very refreshing. They are of a light yellow colour, nearly round, and about half an inch in diameter; the volatile oil of the rind was not at all disagreeable.

At this time Leichhardt was in the region to the west of the Expedition Range at about latitude 24° 45′ S., in what is now Denison County.

About 10 years later Ferdinand von Müller accompanied the North Australian Expedition, under the command of A. C. Gregory, and in his report (1858, p. 143) noted that this species is one of the plants characteristic of the Brigalow Scrub. He called the fruit "a small lemon" and stated that it was among the native fruits eaten by the exploring party.

Prof. J. H. Maiden (1888, p. 489; 1889, p. 8), Colonial Botanist of New South Wales, Australia, said of this species:

> 'Native Kumquat,' 'Desert Lemon.'
> The fruit is globular, and about half-an-inch in diameter. It produces an agreeable beverage from its acid juice. A fair preserve may be made out of the fruit.

Prof. F. M. Bailey (1895, p. 17) wrote as follows:

> *Atalantia glauca*, J. D. Hook. The Kumquat or Lime of our Downs country. Order Rutaceæ, and closely allied to the orange; often attains the size of a small tree, but while only the size of a small shrub yields a great abundance of fruit, which is gathered and converted into jam by the settlers. By careful selection and cross-fertilization from this might be obtained varieties worthy of cultivation for the sake of their fruit.

It is evident from this testimony that the Australian desert kumquat when growing in a wild state yields fruits which, though small, are eaten and even prized by the settlers.

This is also shown by the following account, from a letter of F. S. Carne, dated Fairfield, Roma, Queensland, February 15, 1914, addressed to W. M. Carne, of Sydney, Australia, and by him sent to the author.

> * * * The limes flower in the early spring and the fruit is ripe about the end of November. They are to be found principally on broken Brigalow plains in chocolate

soil, mostly shallow and often stony, and seem to have a tendency to form small scrubs covering an acre or so, but in such cases do not bear much fruit. The trees that are growing in small clumps with a fair amount of room between them fruit very heavily and every season. I don't think they bear till at least 5 years old. The trees when mature average about 10 or 12 feet in height and the stem about 6 in. in diameter and about 6 feet from the ground to the limbs. The young trees are very thorny and have very few leaves, but the grown trees are nice and bushy with scarcely any thorns. At certain times of the year they exude a gum of which some people are very fond. It has a slightly tart taste. Although all the grown trees appear of the same kind to me, odd ones have a fruit much larger and the shape of a Lisbon lemon, the fruit of the majority, however, is the shape of the Mandarin. * * * I am sending a bottle of lime jam. We make it every year. * * * Some folk also make pickles of them, and the children use a lot of them for drinks.

(The jam is of the bitter marmalade character and in my opinion a very pleasant novelty. W. M. C[arne].)

Doubtless if this tree were cultivated, fertilized, and irrigated in accordance with the best methods of modern citriculture, fruits of a larger size and of better flavor could be obtained.

It is to be hoped that the botanists and horticulturists of Australia will give more attention to this remarkable species, one of the most interesting of their citrous fruits. This should result in bringing to light unusually hardy, drouth-resistant, large-fruited or otherwise interesting forms.

Even in its present form the Australian desert kumquat is well worthy of culture in regions slightly too dry or too cold to permit ordinary citrous fruits to succeed.

## INTRODUCTION OF EREMOCITRUS GLAUCA IN THE UNITED STATES

Through the cooperation of the Office of Foreign Seed and Plant Introduction, seeds of *Eremocitrus glauca* were secured from Queensland, Australia, in April, 1911. On germination the seedlings showed hypogeous cotyledons, succeeded by very slender cataphylls.[1] Very soon long, slender spines were produced near the axil of each leaf. In spite of every effort to force these young seedlings into a vigorous growth, it has proved impossible in the three years that have elapsed to induce the formation of any full-sized leaves such as are seen on specimens collected in Australia. These plants continue to produce cataphylls and very long, slender spines. Even in Australia the trees when young are very thorny and have only very narrow leaves.

Plants have been sent to a number of localities in Florida, Alabama, Louisiana, Texas, New Mexico, Arizona, California, and Oregon for trial and within a year or two it will be possible to ascertain with a fair degree

[1] The Australian species of Citrus, *C. australis*, *C. australasica*, and *C. Garrowayi*, germinate in the same way, producing alternate cataphylls which gradually become larger and usually after a few months are replaced by true leaves of nearly full size. These three Australian species of Citrus, together with the broad-leaved *C. inodora* F. M. Bail., constitute a very distinct group of species differing from the other species of Citrus in a number of other characters, such as the small flowers, with free stamens and a few-celled ovary with only four or six ovules in a cell.

of accuracy the soil and climatic factors which limit the culture of the desert kumquat in the United States.

## THE UTILIZATION OF EREMOCITRUS IN BREEDING

From the taxonomic study of Eremocitrus, it is clear that its nearest relationship is with the peculiar Australian species of Citrus (especially *C. australis* and *C. australasica*), with which it shows close similarities in many characters of fundamental importance, such as the flower and fruit structure and the method of germination.

This close relationship, deduced from the botanical characters, is confirmed by the fact that *Eremocitrus glauca* grows vigorously when grafted on *Citrus australasica* (Pl. VIII, fig. 1) and that *Citrus australasica* grafts readily on *Eremocitrus glauca*. It has been found that *Citrus australasica* hybridizes freely with at least two cultivated species of Citrus, [1] and it is not only almost certain that *Eremocitrus glauca* will cross with *Citrus australasica* and the other Australian species of Citrus, but also very probable that it will hybridize with the commonly cultivated Asiatic species of Citrus.

The desert kumquat, native to the semiarid Australian scrubs, able to withstand severe cold in winter as well as burning heat and extreme dryness both of the soil and air in summer, is the most promising species known for use in breeding new types of hardy citrous fruits. Every effort is being made to hasten its flowering, so that hybrids can be made, using it as one of the parents. The fact that the desert kumquat has edible fruits without any disagreeable acrid oil in the peel or in the juice makes it far more promising than the Chinese trifoliate orange, *Poncirus trifoliata* (L.) Raf. (=*Citrus trifoliata* L.), for breeding hardy citrous fruits for table use.

The discovery of this markedly drouth-resistant species in the Australian scrubs opens the way to the breeding of a new class of citrous fruits, able to grow with much less water than is required by ordinary oranges, lemons, or grapefruits.

## GRAFTING AND BUDDING EREMOCITRUS

The Australian desert kumquat can be readily grafted or budded on all of the commonly cultivated species of Citrus, such as the orange, grapefruit, lemon, etc., and also on the Australian finger lime (*C. australasica*). It grows very well on the tabog of the Philippine Islands (*Chaetospermum glutinosa* (Blanco) Swing.), and on the wood-apple of India (*Feronia elephantum* Corr.). (See Pl. VIII.)

The various species of Citrus graft easily on Eremocitrus, which makes it possible to test this new hardy stock for types of soil to which

[1] Mr. George W. Oliver crossed *Citrus australasica* with *C. mitis* in 1909, and the writer crossed the same species with *C. aurantifolia*, the common lime, in 1913, in the greenhouses of the Department of Agriculture at Washington, D. C. Vigorous hybrids were secured from both of these crosses.

the commonly used citrous stocks are not well adapted. It is not impossible that the desert kumquat, being adapted to grow in desert soils, which are usually more or less saline, will prove able to withstand more "alkali" in the soil than the Asiatic species of Citrus, which are indeed very sensitive to salty soils or water.

Being different from Citrus in so many visible characters, it is possible that Eremocitrus will also differ physiologically and prove resistant to some of the many fungous diseases that attack citrous stocks.

## NEED FOR TAXONOMIC STUDY OF THE WILD RELATIVES OF CULTIVATED PLANTS

The bringing to light of the true relationships and possible uses of Eremocitrus, which, although described 66 years ago, has remained to this day practically unknown to botanists and horticulturists, is another link in the chain of arguments going to prove that a better knowledge of the wild relatives of our crop plants is indispensable as a preparation for their improvement by breeding.

It is certainly surprising that a plant so remarkable as *Eremocitrus glauca*, closely related to our cultivated citrous fruits and bearing edible fruits in a wild state, the only desert plant known in the whole orange subfamily, and the hardiest of all the evergreen species, has never before been introduced into culture or utilized in breeding experiments.

Probably the neglect of this remarkably interesting plant in the past has been due largely to the unfortunate nomenclatorial history of the species. Originally placed in Triphasia, it would naturally be supposed to be similar to *Triphasia trifolia* (Burm.) Wilson, common in gardens in tropical and subtropical countries. This plant is a small shrub with trifoliate, almost stalkless, leaves, subtended by paired spines. The fruit is a little berry filled with a sweetish and aromatic mucilaginous pulp, very unlike an orange and not at all closely related to the genus Citrus.

After being classed as a species of Triphasia for 15 years, the Australian desert kumquat was removed to the genus Atalantia by Bentham in 1863. It is true that this placed it in a genus more closely allied to Citrus than is Triphasia, but about this time Baillon's view that Citrus is closely related to the Bael fruit (*Aegle marmelos* (L.) Corrêa) and the wood-apple of India began to be accepted generally by botanists, and as a result the possibility of a species of Atalantia being closely allied to Citrus seems not to have occurred to any of the botanists who have published concerning this group of plants during the past third of a century.

That the discovery of Eremocitrus is not a unique result of some extraordinary good fortune is shown by the fact that an equally striking and equally misunderstood new genus, Citropsis (Swingle and Keller-

man, 1914), also closely related to Citrus, has lately been described from tropical Africa and also by the recent discovery of a new and curious species of Citrus (Swingle, 1913), growing wild at higher altitudes and farther north than any other previously known species of the genus.

The citrous fruits are perhaps the most interesting and in many ways the best known of all our fruits, so that the surprising lack of knowledge as to the wild relatives of Citrus is doubtless duplicated in the case of many other commonly cultivated plants. Until these neglected botanical resources are brought to light and rendered available to the experimenter by critical taxonomic study it is not possible to undertake the improvement of our crop plants in the most rational and effective way. This hybridizing of our crop plants with their wild relatives is a work of the greatest moment at the present time, when the constantly increasing cost of food directs attention to the necessity of extending agriculture by bringing under culture lands too dry, too wet, too salty, too poor, or situated in climates too hot, too cold, or too variable for the culture of the crop plants as they now exist.

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## PLATE VIII

*Eremocitrus glauca* (Lindl.) Swing. S. P. I. No. 29660. Grown from seed from the Condamine River near Chinchilla, Queensland, Australia. One-eighth natural size.

Fig. 1.—Desert kumquat grafted on the Australian finger lime (*Citrus australasica*).
Fig. 2.—Desert kumquat grafted on the wood-apple (*Feronia elephantum*). Two years' growth.


1
2

# RELATION OF BACTERIAL TRANSFORMATIONS OF SOIL NITROGEN TO NUTRITION OF CITROUS PLANTS

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It is practically certain that there are a number of immediate causes for the decrease in the yield of fruit and for the general physiological decadence of citrous trees apparent in small areas scattered throughout the orange belt in California. In this region, as in many irrigated orchard districts of both deciduous and citrous trees, a high yield of fruit is correlated with a very luxuriant and vigorous vegetative growth of the individual trees. To an observer accustomed to the orchards of the humid regions of the East, this simultaneous enhancement of both the reproductive and vegetative functions of the plant suggests an abnormality due to a general physiological stimulation slightly in excess of the optimum caused by high temperature, excessive insolation, extreme fluctuations in quantity of soil moisture, and an abundant, though perhaps erratic, supply of soil nutrients. Under such conditions a very slight additional stimulus might cause rapid deterioration or injury. While this premise is not essential to the establishment of a relation between changes in soil nitrogen and abnormal nutrition, to recognize this unusual parallel of functions as signalling distinct overstimulation gives a logical reason for expecting similar or identical symptoms of malnutrition in different citrous groves, even though the immediate causes of their decadence within different limited areas might differ widely.

Extensive bacteriological studies upon soils near Riverside, Cal., and occasional examinations of soils of other irrigated regions, together with observations upon the physiology of citrous trees and physiological experiments upon citrous seedlings, are believed to offer defensible hypotheses for advising the employment of certain methods of crop culture to ameliorate at least a portion of the baffling trouble in southern California generally known as citrous malnutrition, chlorosis, or mottle leaf (Smith and Smith, 1911).[1]

A careful examination of the soluble-salt content[2] of soil samples taken throughout southern California showed in common with other investigators (Hilgard, 1906b; Swingle, 1904; Loughridge, 1903, 1911;

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 113.

[2] Except for the estimation of nitrates, determinations were made in accordance with the methods described by Schreiner, O., and Failyer, G. H., Colorimetric, turbidity, and titration methods used in soil investigations, U. S. Dept. of Agr., Bur. Soils Bul. 31. Nitrates were determined by a modification of the Thieman-Schultze method, for explanation of which see Kellerman, K. F., and Smith, N. R., The absence of nitrate formation in cultures of Azotobacter, Cent. f. Bakt. [etc.], Abt. 2, Bd. 40, No. 19/21, p. 479–482.

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Means and Holmes, 1902, p. 601) a wide variation in the percentage of carbonates, chlorids, nitrates, and sulphates, chiefly of sodium, potassium, calcium, and magnesium, which are the salts usually present in appreciable quantities. From a casual examination of these data there appears to be no relation between soils of high salt content and malnutrition of trees. In figures 1, 2, and 3, however, it is evident that when adjacent areas are compared, the soils from the vicinity of deteriorating trees are richer in nitrate nitrogen. The quantities of other salts show no constant relationship to good or poor areas. Except for a slight increase in the quantity of bicarbonates during the spring, there is no evidence of seasonal

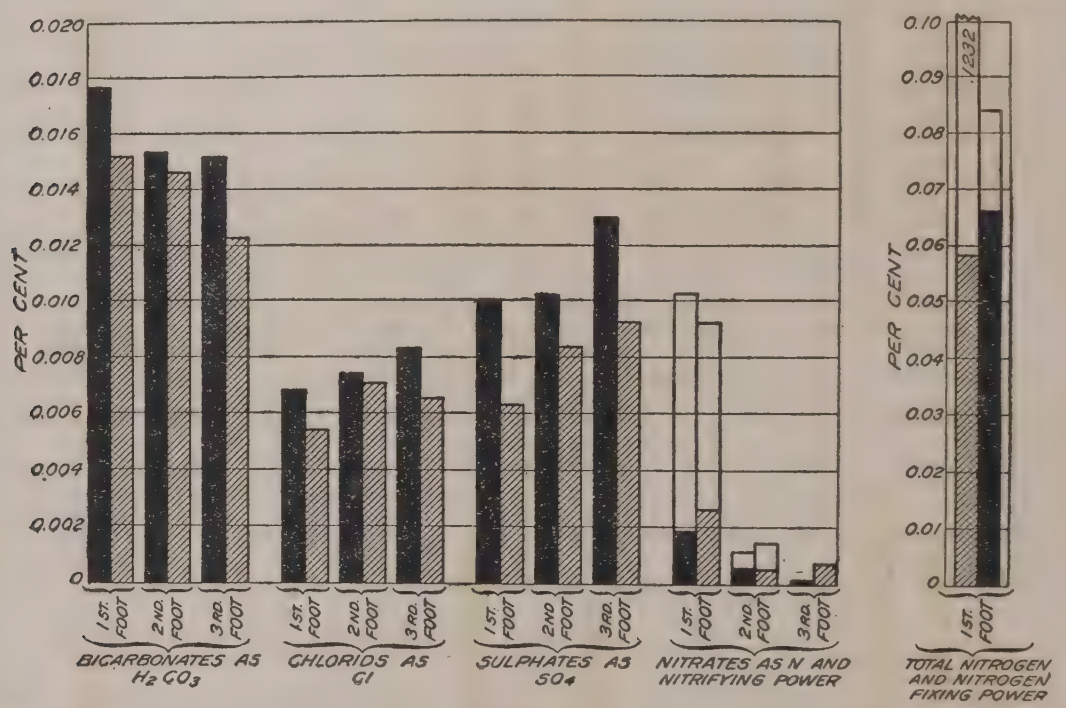



FIG. 1.—Diagram showing relative nitrogen-fixing power, nitrifying power, and salt content of soil samples from good and poor areas in an orange grove which was unproductive and markedly chlorotic throughout. The good areas were themselves very unproductive.[1] Black columns show good areas; hatched columns indicate poor areas.

variation for the various salts. Though decided variations in salt content of the soil were observed occasionally, they were evidently due to the infrequent rains.

The nitrate content of the soil of so-called "poor" areas is not excessive unless the crust analyses shown in figure 4 are taken into account. During irrigation it is obvious that in the poor areas the numerous shallow lateral roots (Mills, 1902) will be bathed in a solution which temporarily is relatively stronger than during the intermediate periods. The reports that in some cases applications of fertilizers high in nitrogen to orange groves showing symptoms of malnutrition have accelerated instead of retarded the deterioration offer corroborative evidence of the danger of an excessive supply of readily available nitrogen. It is, of

[1] The samples were collected near Riverside, Cal., in June, July, August, and October, 1912, and February, March, and June, 1913. The averages of the analyses are given.

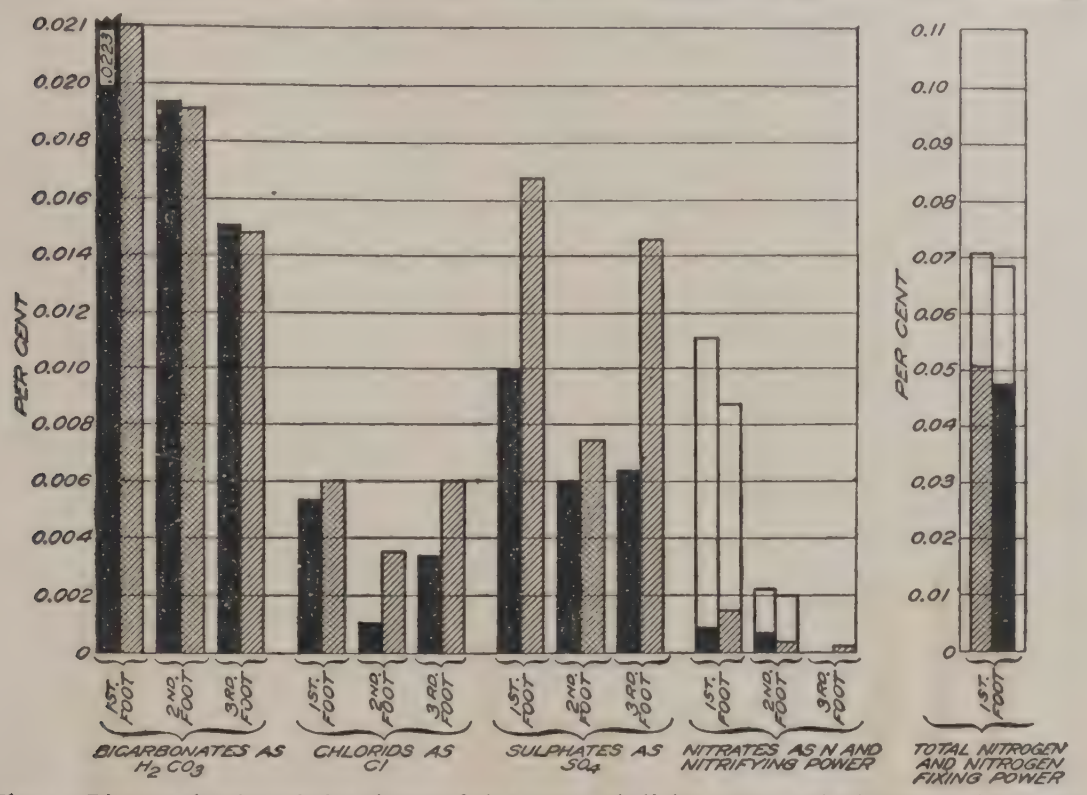



FIG. 2.—Diagram showing relative nitrogen-fixing power, nitrifying power, and salt content of good and poor areas in an orange grove that produced a fair crop of marketable fruit. The poor areas were unproductive.[1] Black columns show good areas; hatched columns indicate poor areas.

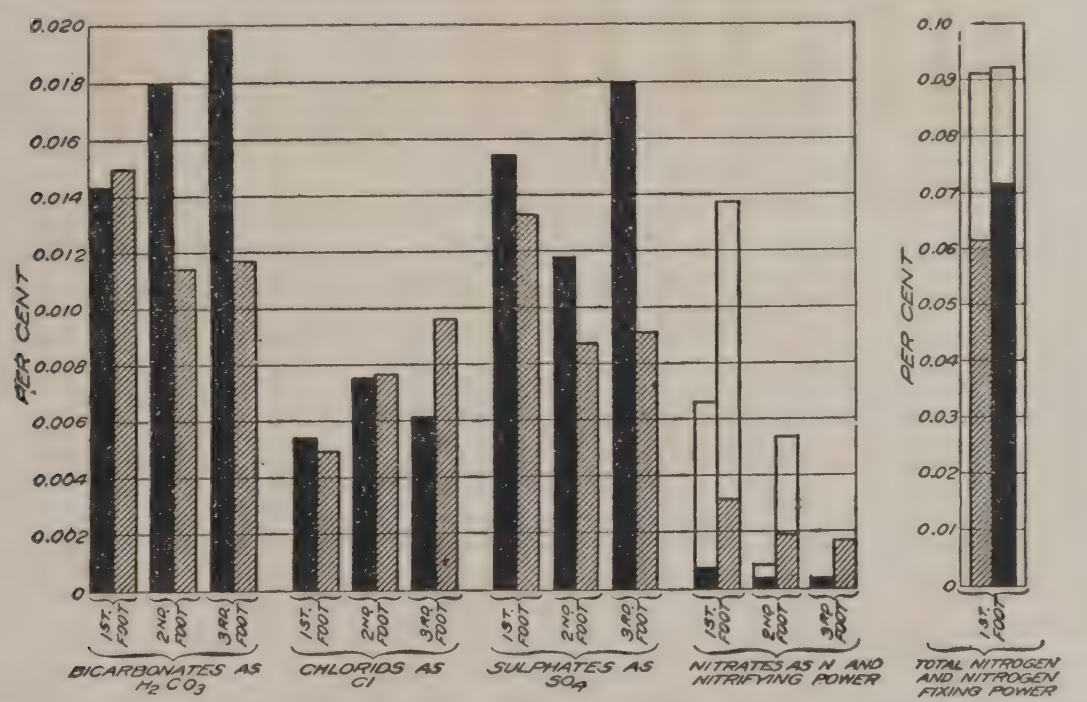



FIG. 3.—Diagram showing relative nitrogen-fixing power, nitrifying power, and salt content of soil samples from good and poor areas in an orange grove which showed decided symptoms of chlorosis associated with small yields of low-grade fruit. There was but a slight difference in the vigor of the orange trees [2] growing upon the good and the poor areas.[3] Black columns show good areas; hatched columns indicate poor areas.

[1] The samples were collected near Riverside, Cal., in June, July, August, and October, 1912, and February, March, and June, 1913. The averages of the analyses are given.

[2] The trees of this grove were considered as probably unusually sensitive, because the stock, originally budded to lemons, was later budded to oranges, thus leaving a portion of the lemon trunk in the trees.

[3] The samples were collected near Riverside, Cal., in October, 1912, and February, March, and June, 1913. The averages of the analyses are given.

course, recognized that records of fertilizer practice in California are meager and unsatisfactory. Somewhat extensive greenhouse experiments have been carried on in Washington, and although laboratory results can not be directly translated into field conditions, the results again indicate that nitrate nitrogen in excess produces the typical symptoms of malnutrition. In the experiments reported in detail in Tables I, II, and III both greenhouse soil and virgin California soil from typical orange-growing areas were used. Moderate quantities of nitrate gave no apparent result, but it has been noted in earlier publications (Moore and Kellerman, 1905) that toxic limits established in the laboratory may be expected to be much higher than will be found to exist under a natural and favorable environment. If the hypothesis of overstimulation in southern California is accepted, a wide variation between the low inhibiting or toxic limits in the field and comparatively high toxic limits found in the greenhouse in Washington is therefore to be expected.

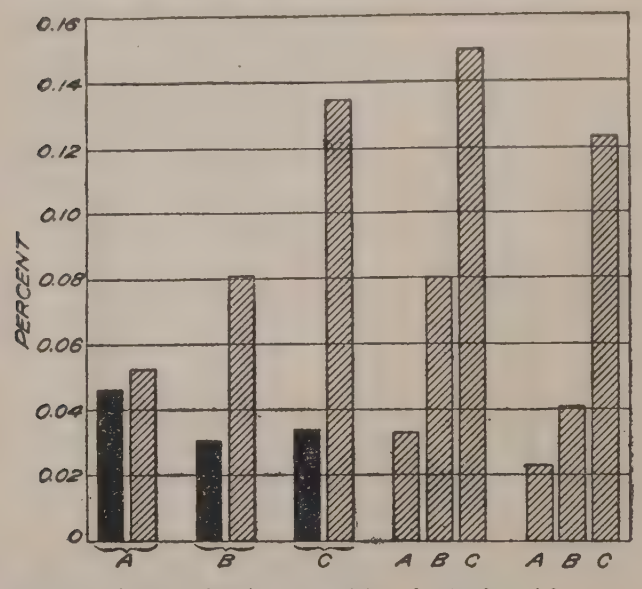



FIG. 4.—Diagram showing quantities of salts found in crust samples of soil from the same areas illustrated in figures 1, 2, and 3.

TABLE I.—*Effect of certain salts and combinations of salts on grapefruit seedlings growing in greenhouse soil at the Department of Agriculture, Washington, D. C.*[a]

| Laboratory No. | Soil treatment. | Condition of trees after 74 days. | Condition of trees after 144 days. |
|---|---|---|---|
| 770 | Check | Normal | Normal. |
| 771 | do | do | Do. |
| 772 | $CaCO_3$ (5 per cent) | do | Slight leaf burning (?). |
| 773 | do | do | Normal. |
| 774 | N as $KNO_3$ (0.04 per cent) | Leaves shed | Leaves shed. |
| 775 | do | Leaves burning | Leaves burning. |
| 776 | $CaCO_3$ (1 per cent) + N as $KNO_3$ (0.04 per cent). | Leaves shed | New leaves; chlorotic. |
| 777 | do | do | Do. |
| 778 | Cl as NaCl (0.04 per cent) | Normal | Leaves burning. |
| 779 | do | do | Do. |

[a] Grapefruit seedlings 2 years and 4 months old, about 24 inches in height, were transplanted into 6-inch pots of soil mixture consisting of equal parts of sand and sod composted in rather stiff clay soil.

TABLE I.—*Effect of certain solts and combinations of salts on grapefruit seedlings growing in greenhouse soil at the Department of Agriculture, Washington, D. C.*—Con.

| Laboratory No. | Soil treatment. | Condition of trees after 74 days. | Condition of trees after 144 days. |
|---|---|---|---|
| 780 | Cl as NaCl (0.04 per cent) + N as $KNO_3$ (0.04 per cent). | Leaves shed | Few new leaves; chlorotic. |
| 781 | .....do | .....do | Do. |
| 782 | $CaCO_3$ (1 per cent) + Cl as NaCl (0.04 per cent). | Normal | Normal. |
| 783 | .....do | .....do | Do. |
| 784 | HCO as $NaHCO_3$ (0.04 per cent). | .....do | Do. |
| 785 | .....do | .....do | Do. |
| 786 | $HCO_3$ as $NaHCO_3$ (0.04 per cent) + N as $KNO_3$ (0.04 per cent). | Leaves shed | Do. |
| 787 | .....do | .....do | New leaves normal. |
| 788 | $SO_4$ as $Na_2SO_4$ (0.04 per cent) + N as $KNO_3$ (0.04 per cent). | Normal | Leaves nearly all shed. |
| 789 | .....do | .....do | Do. |

TABLE II.—*Effect of certain salts and combinations of salts on sour-orange seedlings growing in California soil at Washington, D. C.*[a]

| Laboratory No. | Soil treatment. | Condition of trees after 26 days. | Condition of trees after 46 days. | Condition of trees after 90 days. |
|---|---|---|---|---|
| 930 | Check | Normal | Normal | Normal; growing. |
| 931 | .....do | .....do | .....do | Do. |
| 932 | $CaCO_3$ (1 per cent) | .....do | .....do | Do. |
| 933 | .....do | .....do | .....do | Do. |
| 934 | $CaCO_3$ (3 per cent) | .....do | .....do | Do. |
| 935 | .....do | .....do | .....do | Do. |
| 936 | $CaCO_3$ (10 per cent) | .....do | .....do | Do. |
| 937 | .....do | .....do | .....do | Do. |
| 938 | N as $KNO_3$ (0.02 per cent)[b] | .....do | .....do | Do. |
| 939 | .....do.[c] | .....do | .....do | Do. |
| 940 | N as $KNO_3$ (0.04 per cent)[d] | Dead; replanted | Dead | Dead. |
| 941 | .....do.[e] | Normal | Normal | Subnormal. |
| 942 | N as $KNO_3$ (0.06 per cent)[f] | Dead; replanted | Dead | Dead. |
| 943 | .....do.[g] | .....do | .....do | Do. |
| 944 | Cl as KCl (0.02 per cent) | Normal | Normal | Subnormal. |
| 945 | .....do | .....do | .....do | Normal; growing. |

[a] Sour-orange seedlings 58 days old were transplanted into 4-inch paraffined pots containing virgin soil taken from near Riverside, Cal. Plants were watered with distilled water. All drainage water was caught and returned to each pot, thus preventing any change in salt content.

Percentage of nitrates present after 90 days: [b] 0.0018 per cent. [c] 0.0061 per cent. [d] 0.0223 per cent. [e] 0.0087 per cent. [f] 0.0200 per cent. [g] 0.0194 per cent.

TABLE II.—*Effect of certain salts and combinations of salts on sour-orange seedlings growing in California soil at Washington, D. C.*—Continued.

| Laboratory No. | Soil treatment. | Condition of trees after 26 days. | Condition of trees after 46 days. | Condition of trees after 90 days. |
|---|---|---|---|---|
| 946 | Cl as KCl (0.05 per cent) | Dead; replanted | Dead | Dead. |
| 947 | do | Normal | Subnormal | Do. |
| 948 | Cl as KCl (10 per cent) | Dead; replanted | Dead | Do. |
| 949 | do | Normal | do | Do. |
| 950 | N as $KNO_3$ (0.04 per cent) + $CaCO_3$ (1 per cent).[h] | do | Burning | Subnormal. |
| 951 | do.[i] | Dead; replanted | Normal | Do. |
| 952 | N as $KNO_3$ (0.04 per cent) + $CaCO_3$ (3 per cent).[j] | do | Burning | Normal; growing. |
| 953 | do.[k] | Normal | Dead | Dead. |
| 954 | N as $KNO_3$ (0.04 per cent) + $CaCO_3$ (10 per cent).[l] | Dead; replanted | Normal | Normal; growing. |
| 955 | do | Normal | do | Subnormal. |
| 956 | N as $KNO_3$ (0.04 per cent) + Cl as KCl (0.02 per cent). | do | Dead | Dead. |
| 957 | do | do | Burning | Do. |
| 958 | N as $KNO_3$ (0.04 per cent) + Cl as KCl (0.05 per cent).[o] | do | Dead | Do. |
| 959 | do.[p] | do | do | Do. |
| 960 | N as $KNO_3$ (0.02 per cent) + Cl as KCl (0.02 per cent).[m] | Dead; replanted | do | Normal; growing. |
| 961 | do.[n] | do | do | Do. |
| 962 | Cl as KCl (0.05 per cent) + Ca $CO_3$ (3 per cent). | do | do | Do. |
| 963 | do | do | do | Do. |
| 964 | Cl as KCl (0.05 per cent) + $CaCO_3$ (10 per cent). | do | do | Subnormal. |
| 965 | do | do | do | Do. |
| 966 | Cl as KCl (0.02 per cent) + $CaCO_3$ (3 per cent). | Normal | Normal | Normal; growing. |
| 967 | do | do | do | Do. |

Percentage of nitrates present after 90 days: [h] 0.0095 per cent. [i] 0.0035 per cent. [j] 0.0046 per cent. [k] 0.0238 per cent. [l] 0.00485 per cent. [m] 0.0012 per cent. [n] 0.0017 per cent. [o] 0.0081 per cent. [p] 0.0130 per cent.

TABLE III.—*Effect of certain salts and combinations of salts on grapefruit seedlings growing in California soil at Washington, D. C.*[a]

| Laboratory No. | Soil treatment. | Condition of trees after 22 days. | Condition of trees after 48 days. | Condition of trees after 88 days. |
|---|---|---|---|---|
| 892 | Check | Normal | Normal | Normal; growing. |
| 893 | do | do | do | Do. |
| 894 | $CaCO_3$ (1 per cent) | do | do | Do. |
| 895 | do | do | do | Do. |
| 896 | $CaCO_3$ (3 per cent) | do | do | Do. |
| 897 | do | do | do | Do. |
| 898 | $CaCO_3$ (10 per cent) | do | do | Do. |
| 899 | do | do | do | Do. |
| 900 | N as $KNO_3$ (0.02 per cent)[b] | do | do | Do. |
| 901 | do.[c] | do | do | Do. |
| 902 | N as $KNO_3$ (0.04 per cent)[d] | Dead; replanted | Dead | Dead. |
| 903 | do.[e] | do | Normal | Do. |
| 904 | N as $KNO_3$ (0.06 per cent)[f] | Normal | Dead | Do. |
| 905 | do.[g] | Dead; replanted | do | Do. |
| 906 | Cl as KCl (0.02 per cent) | Normal | Normal | Normal; growing. |
| 907 | do | do | do | Do. |
| 908 | Cl as KCl (0.05 per cent) | do | Dead | Dead. |
| 909 | do | Dead; replanted | do | Do. |
| 910 | Cl as KCl (10 per cent) | Normal | do | Do. |
| 911 | do | do | do | Do. |
| 912 | N as $KNO_3$ (0.04 per cent) + $CaCO_3$ (1 per cent).[h] | Dead; replanted | do | Do. |
| 913 | do.[i] | Normal | do | Do. |
| 914 | N as $KNO_3$ (0.04 per cent) + $CaCO_3$ (3 per cent).[j] | do | do | Do. |
| 915 | do.[k] | do | do | Do. |
| 928 | N as $KNO_3$ (0.04 per cent) + $CaCO_3$ (10 per cent).[l] | Dead; replanted | Subnormal | Normal; growing. |
| 929 | do.[m] | do | do | Do. |
| 924 | N as $KNO_3$ (0.02 per cent) + Cl as KCl (0.02 per cent).[n] | Normal | Dead | Dead. |
| 925 | do.[o] | do | do | Do. |
| 920 | N as $KNO_3$ (0.04 per cent) + Cl as KCl (0.02 per cent).[p] | Dead; replanted | do | Do. |
| 921 | do.[q] | do | Normal | Subnormal. |

[a] Grapefruit seedlings 3 months old were transplanted into paraffined pots containing virgin soil taken near Riverside, Cal. Plants were watered with distilled water. All drainage water was caught and returned to each pot, thus preventing any change in salt content.

Percentage of nitrates present after 88 days: [b] 0.0019 per cent. [c] 0.0018 per cent. [d] 0.0117 per cent. [e] 0.0041 per cent. [f] 0.0200 per cent. [g] 0.0205 per cent. [h] 0.0223 per cent. [i] 0.0270 per cent. [j] 0.0157 per cent. [k] 0.0189 per cent. [l] 0.0029 per cent. [m] 0.0029 per cent. [n] 0.0023 per cent. [o] 0.0044 per cent. [p] 0.0213 per cent. [q] 0.0091 per cent.

TABLE III.—*Effect of certain salts and combinations of salts on grapefruit seedlings growing in California soil at Washington, D. C.*—Continued.

| Laboratory No. | Soil treatment. | Condition of trees after 22 days. | Condition of trees after 48 days. | Condition of trees after 88 days. |
|---|---|---|---|---|
| 922 | N as $KNO_3$ (0.04 per cent) + Cl as KCl (0.05 per cent).[r] | Normal | Subnormal | Subnormal. |
| 923 | do.[s] | Dead; replanted | Dead | Dead. |
| 926 | Cl as KCl (0.02 per cent) + $CaCO_3$ (3 per cent). | Normal | Normal | Normal; growing. |
| 927 | do | do | do | Subnormal. |
| 916 | Cl as KCl (0.05 per cent) + $CaCO_3$ (3 per cent). | do | Dead | Dead. |
| 917 | do | do | do | Do. |
| 918 | Cl as KCl (0.05 per cent) + $CaCO_3$ (10 per cent). | do | Normal | Do. |
| 919 | do | do | do | Do. |

Percentage of nitrates present after 88 days: [r] 0.0042 per cent. [s] 0.0275 per cent.

It should be noted also that the virgin California soil used in these experiments would naturally endure greater additions of nitrate salts before the toxic limit of orange plants would be reached than would the soils of the older groves. In the latter soils there has been an appreciable accumulation of soluble salts, and, as will be shown later, this would intensify the effect of high nitrates.

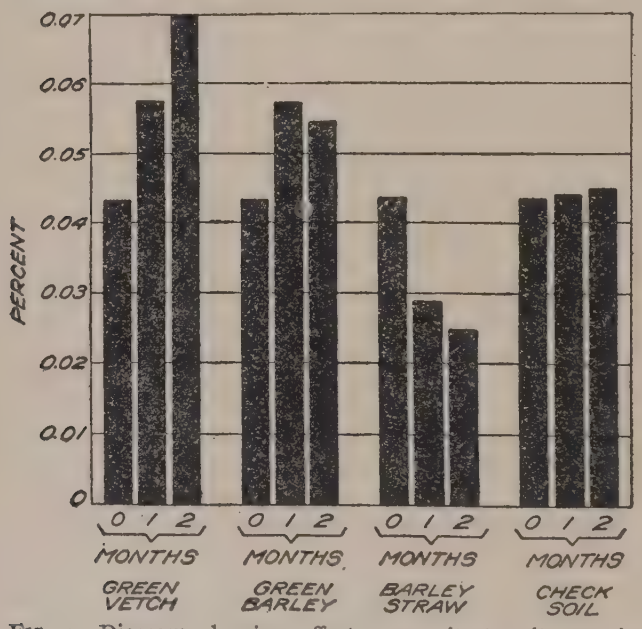



FIG. 5.—Diagram showing effect upon nitrate nitrogen in samples of California soil of additions of 2 per cent of green vetch, green barley, and mature barley straw.

As will be seen by an examination of the analyses of nitrate nitrogen made at the close of several experiments, a considerable though rather erratic decrease of this salt took place, and it is probable that this denitrification occurred during the early periods. The toxic limits of the pot experiments therefore are actually between 0.05 and 0.0100 per cent, as against 0.005 to 0.015 per cent in the field, exclusive of crusts. It is evident, therefore, that nitrates are more fatal than chlorids, that nitrates and chlorids together intensify the symptoms of injury, and that moderate quantities of lime—less than 10 per cent of calcium carbonate

(Hilgard, 1906a)—exert a more or less pronounced protective action. Sulphates are comparatively innocuous and are not reported upon. In this connection it should be noted that while, as would be expected from the analogy of California soils with the soils of the citrous regions of Florida, rather large quantities of calcium carbonate are favorable to the citrous plants, even slight traces of calcium oxid or calcium hydrate have an immediate toxic effect. The beneficial effect of calcium carbonate in flocculating the impervious soils and thus facilitating their permeability to irrigation water renders it valuable from a physical as well as a biological standpoint in many areas.

While no adequate data are recorded, records of at least temporary benefit from the application of nitrate fertilizers to chlorotic orange groves are available. Laboratory and greenhouse studies offer a possible explanation of such a phenomenon under certain conditions. As shown in figures 5, 6, and 7, different substances which are commonly plowed under to maintain humus in the soil show pronounced differences in their effect upon the soil bacteria. Mature barley straw or pure cellulose rapidly reduces the nitrate content of a soil [1] and may eliminate it entirely if it is present in relatively small quantities; furthermore, although but little nitrogen is actually lost, it seriously delays the renitrification into nitrate. Green manures, on the other hand, while causing some actual loss of total nitrogen, do not materially disturb the ratios of the nitrogenous compounds. The same order of limitation is shown by these substances in their effect upon the nitrification of pepton in the soil. As shown in Tables IV and V, seedling citrous plants were actually forced by the addition of cellulose and of straw into an apparently typical state of malnutrition through nitrogen starvation caused by the unbalancing of the soil flora and the utilization of all of the nitrate by the organisms which decompose straw and cellulose.[2]

[1] From unpublished results in 1911. Any mature straw, as well as pure cellulose, causes a rapid denitrification of soil nitrate, and the subsequent nitrification which again forms nitrate is very slow. (*See also* Lipman, J. G., and Brown, Percy E., 1909.)

[2] From unpublished results in 1907, showing injury to corn by the bacterial denitrification of nitrate to nitrite, it was supposed that nitrite poisoning might be the cause of the injury to citrous plants in the conditions shown in Table V. The presence of appreciable quantities of nitrite in the soil supporting apparently normal citrous plants indicates that complete elimination of nitrate is more injurious to them than is the formation of slight quantities of nitrite.

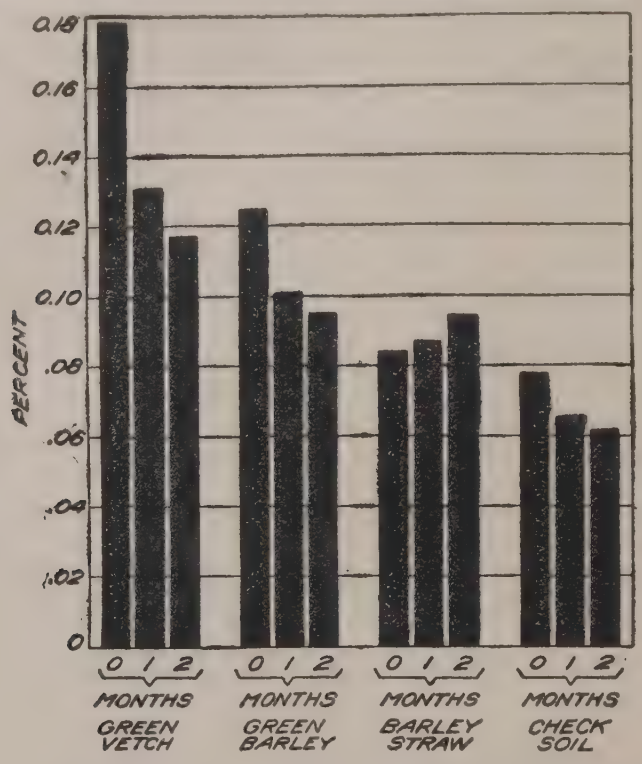



FIG. 6.—Diagram showing effect upon total nitrogen, not including nitrate nitrogen, in samples of California soil of additions of 2 per cent of green vetch, green barley, and mature barley straw.

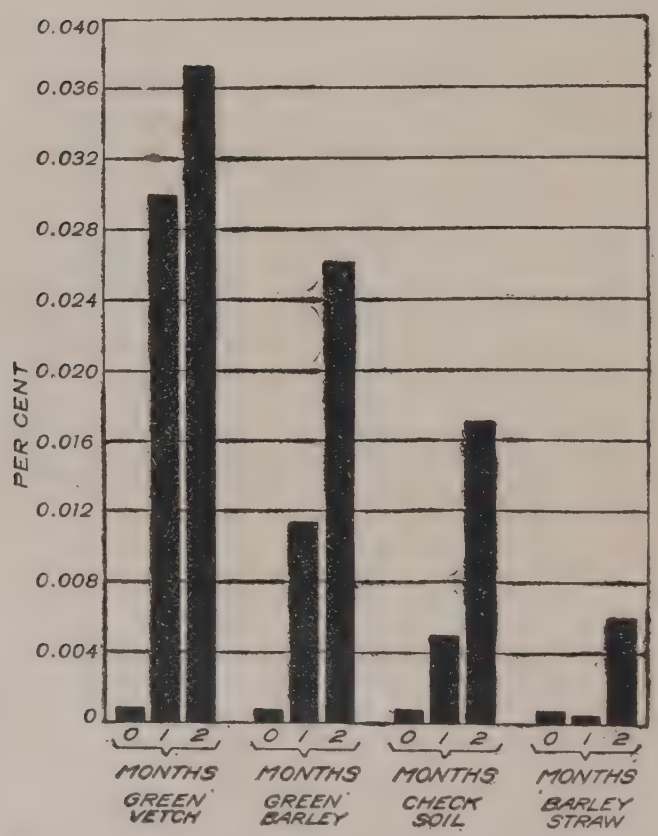



FIG. 7.—Diagram showing effect of additions of 2 per cent of green vetch, green barley, and mature barley straw upon the nitrifying power of samples of California soil.

TABLE IV.—*Effect of green manure, straw, pure cellulose, and nitrate on grapefruit seedlings growing in greenhouse soil at the Department of Agriculture, Washington, D. C.*[a]

| Laboratory No. | Soil treatment. | Condition of trees after 19 days. | Condition of trees after 57 days. | Condition after 166 days. |
|---|---|---|---|---|
| 734 | Check | Normal | Normal | Normal. |
| 735 | do | do | do | Do. |
| 736 | $KNO_3$ | Slightly chlorotic | do | |
| 737 | do | do | do | |
| 738 | Wheat straw (1 per cent). | do | Slightly yellowish | Yellow and spotted. |
| 739 | do | Normal | do | Do. |
| 740 | Wheat straw (1 per cent) + $KNO_3$. | Chlorotic | Normal | |
| 741 | do | do | do | |
| 742 | Green sweet clover (1 per cent). | do | do | Normal. |
| 743 | do | do | do | Do. |
| 744 | Green sweet clover + $KNO_3$. | Subnormal | do | |
| 745 | do | Chlorotic | do | |
| 746 | Green devil's grass (1 per cent). | Slightly chlorotic | do | Do. |
| 747 | do | do | do | Do. |
| 748 | Green devil's grass + $KNO_3$. | do | do | |
| 749 | do | Chlorotic | do | |
| 750 | Filter paper (1 per cent). | do | Yellow | Yellow and spotted. |
| 751 | do | do | do | Very yellow and spotted. |
| 752 | Filter paper (1 per cent) + $KNO_3$. | Very chlorotic | Normal | |
| 753 | do | do | do | |

[a] Grapefruit seedlings about 2½ months old were transplanted into 6-inch pots of greenhouse bench soil. All organic materials added were cut fine and 1 per cent added dry-weight basis. Nitrogen as nitrate was added once a week to Nos. 736, 737, 740, 741, 744, 745, 747, 749, 752, and 753 at the rate of 0.01 per cent at each application. The spotted condition of the leaves where straw and filter paper were used seems to be a symptom of malnutrition in an advanced stage. These leaves showed no "veining" after the first few days.

TABLE V.—*Effect of straw and nitrate on sweet-orange, sour-orange, and grapefruit seedlings growing in California soil at Washington, D. C.*[a]

SOUR ORANGE (*Citrus aurantium* L.).

| Laboratory No. | Soil treatment. | Condition of seedlings after 73 days. |
|---|---|---|
| 964 | Check | Normal; growing. |
| 965 | .....do | Do. |
| 966 | Finely ground straw (1 per cent) | Yellowish. |
| 967 | .....do | Do. |
| 968 | Finely ground straw (2 per cent) | Do. |
| 969 | .....do | Do. |
| 970 | Finely ground straw (1 per cent) + N as $KNO_3$ (0.02 per cent) | Normal; growing. |
| 971 | .....do | Do. |
| 972 | Finely ground straw (2 per cent) + N as $KNO_3$ (0.02 per cent) | Do. |
| 973 | .....do | Do. |

SWEET ORANGE (*Citrus sinensis* Osb.).

| | | |
|---|---|---|
| 988 | Check | Normal; growing. |
| 989 | .....do | Do. |
| 974 | Finely ground straw (1 per cent) | Yellow. |
| 975 | .....do | Do. |
| 976 | Finely ground straw (1 per cent) + N as $KNO_3$ (0.02 per cent) | Normal; growing. |
| 977 | .....do | Do. |

GRAPEFRUIT (*Citrus grandis* Osb.).

| | | |
|---|---|---|
| 986 | Check | Normal. |
| 987 | .....do | Do. |
| 978 | Finely ground straw (1 per cent) | Yellow. |
| 979 | .....do | Do. |
| 980 | Finely ground straw (1 per cent) + N as $KNO_3$ (0.02 per cent) | Normal. |
| 981 | .....do | Do. |

[a] Orange and grapefruit seedlings about 2 months old were transplanted into paraffined pots containing virgin soil from near Riverside, Cal.; plants were watered with distilled water; all drainage was caught and returned to each pot, thus preventing any change in salt content.

The destruction of cellulose by molds and bacteria is extremely rapid in both good and poor areas, but no correlation of laboratory results and field conditions has as yet been possible. The total number of bacteria in good and poor areas is erratic and seems without significance.

The total nitrogen content and also the nitrogen-fixing power of the poor soils is slightly above that of the good soils, as shown in figures 1, 2, 3, and 4. Similar data have been reported by Headden (1910) for soils of the irrigated orchard regions of Colorado. Contrary to Headden's

opinion, however, this appears to be without significance, as both good and poor soils usually contain rather large quantities of insoluble nitrogen. The control of the rate of nitrification is, on the other hand, of fundamental importance. In pot experiments a normal rate of nitrification is shown to be possible with a green crop turned under, and it seems reasonable to recommend this practice in the field for maintaining the humus supply of the soil. The evidence of the above experiments suggests that the extensive use of mature straw is to be avoided, though light applications of straw to fields too high in nitrate, probably also with the liberal use of ground limestone, might be advantageous.

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# AROMA OF HOPS: A STUDY OF THE VOLATILE OIL WITH RELATION TO THE GEOGRAPHICAL SOURCES OF THE HOPS

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At the present time the principal method for distinguishing the kind and source of hops is by means of their appearance and odor. This must necessarily be an uncertain and crude method because of the inability of individuals to differentiate accurately between similar odors, and, hence, it can serve only to determine extremes in the quality of the hops. If, therefore, a scientific method based on the actual properties of the hops could be worked out, it would be considerably more satisfactory and accurate. Since the aroma, which is conceded to be an important factor in judging hops, can be readily concentrated in the volatile oil, it was thought that a systematic comparison of the properties of oils distilled from hops obtained from the various hop-producing countries of the world and grown during different seasons would give information which would be valuable not only as a means for judging the quality but also to determine the geographical source of the hops.

## FACTORS WHICH INFLUENCE THE AROMA OF HOPS

Since altitude, light, hygrometric conditions, and the composition of the soil affect the composition of the volatile oils of thyme, lavender, rosemary, and peppermint (Jeancard and Satie, 1909; Bonnier, 1894; Lamothe, 1908; Mossler, 1912)[1], it may be easily seen that hops grown in separated localities under different conditions of climate and soil may be widely different in aroma and may produce volatile oils with distinct and constant variations in their chemical properties. This assumption is practically substantiated by Briant and Meacham (1896), who discuss the influence of climate, ripeness, soil, drying, and general manipulation on the value of hops. The amount of rain and sunshine alters the conditions of ripening, and the different degrees of ripeness have a decided effect upon the quality of the hops. Regarding the influence of soil upon the quality of the product, these authors state:

> The effect of soil is, however, undoubtedly very great, for it comes within our experience that on two farms in the same neighborhood but with different soils the hops grown upon one are year after year of a decidedly superior character both as regards resins and flavor, to those on the other, although both are farmed in similar manner and with practically the same climatic conditions.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 158–159.

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According to Sykes and Ling (1907), it is well known that hops grown in different parts of the world differ in aroma. It is further stated that, since the oil from California hops yields the same compounds as the oil from Bavarian or English hops, the difference in odor must be due to the different proportions in which the constituents exist.

While it can not be said with certainty that the aromatic constituents of hops are useful industrially, yet they are of sufficient importance not to be overlooked. Concerning the use of the volatile oil in determining the value of hops, Chapman (1898, p. 233) observes:

> It is, in the first place, perhaps, the surest guide to the general value of a sample of hops, when reliance is placed upon a physical examination alone * * *. In the selection of hops * * * the essential oil is certainly the constituent to which chief attention should be paid.

Hops with a fine, agreeable bouquet are usually preferred to those with a poorer odor. If, therefore, differences in odor are clearly perceptible in different hops, a comparison of the volatile oils should be most important in determining possible differences in the aromatic quality of the hops. Since the volatile oil is the carrier of the aroma and since the aroma is a factor in judging hops, it should be possible to compare the aroma of one sample of hops with another by means of the volatile oil from each.

## ESTERS AS THE PRINCIPAL ODOR BEARERS

Since esters are important factors in determining the odorous quality of volatile oils, it was thought that perhaps this would be a good point of attack for the comparison of the various hop oils. The very agreeable odor of the oil of hops would indicate the presence of esters, and a preliminary test showed them to be present in considerable quantity.

The ester content is easily measured and may be expressed by the ester number. While this value does not express the exact percentage of any particular ester, yet for comparative purposes it answers equally well. For the purpose, therefore, of making a logical chemical comparison of the several oils, the ester value was adopted as being possibly the constant most likely to show any fluctuation which would have a direct bearing on the aromatic quality.

## PHYSICAL AND CHEMICAL PROPERTIES OF OILS AS A BASIS FOR COMPARISON

The physical properties of volatile oils often show variations sufficient to enable comparisons to be made. The specific gravity of most volatile oils is an important factor in their investigation and is influenced to a considerable extent by the source and condition of the plant from which the oil is distilled, as well as by the nature of the constituents of the oil. Optical rotation is a property of volatile oils which is very important,

being dependent almost entirely upon the character of the chemical constituents of the oils. Refraction, although less important than either specific gravity or optical rotation, is of value, since some relationship is known to exist between the chemical compounds and their refractive power. Another physical property which is largely affected by the character of the constituents of the oil is its solubility in alcohol or in dilute alcohol. The presence of terpenes in oils retards solubility, while oxygenated compounds, such as esters and alcohols, increase it.

The boiling points of the various constituents differ considerably. This property is often utilized in the examination of oils for the purpose of effecting a partial separation of the constituents by fractional distillation. The thoroughness of this separation depends largely upon the form of the distilling flask and upon the rapidity of distillation.

All these physical properties were determined for the various samples of hop oils in the hope of detecting any possible differences which might exist.

For further comparison, acidity and saponification values were also determined, although they are of less importance. The determination of alcohols, while important in many oils, was not feasible in this investigation because of the nature of hop oil. It was found that acetylization would not take place quantitatively, and, hence, a measurement of the alcohol content was impossible.

An approximate comparison of the terpene and sesquiterpene content was made possible from the fractionation experiments.

Inasmuch as there is no direct method of assay available which is applicable to oil of hops, it was thought that a careful determination of certain physical and chemical properties of different hop oils would yield data from which a logical comparison could be made. All of the above-mentioned physical and chemical constants are likely to vary with the different conditions of climate, soil, cultivation, ripening, and curing of the hops.

## GENERAL PLAN OF COMPARISON OF VARIOUS HOPS AND HOP OILS

In order to learn whether any constant differences exist in the various kinds of hops, it was planned to compare the oils distilled from hops grown in the hop-producing sections of the United States with the oil distilled from an authentic sample of imported hops, all the samples to be from hops grown during the same season. The sections chosen in the United States were California, Oregon, Washington, and New York. The imported hops were from Bohemia.

A comparison of oils obtained from the hops during a single season would give results which would be valuable in determining differences for that particular season, but it was of the most importance to ascertain whether the same differences occurred from year to year. Therefore the experiments were carried on for four successive seasons, and

the oils obtained from the hops of any one locality were compared with those from the same locality during these years. This procedure permitted an absolutely fair comparison by which similarities or differences in the properties of the oils could be easily followed and any fluctuations readily noted.

## DISTILLATION OF THE VOLATILE OILS

The usual method of steam distillation was applied for the extraction of the volatile oils from the various samples of hops. The conditions of distillation were practically identical in all cases, each sample being distilled until no more oil was noticeable. From 100 to 200 pounds of California, Oregon, and Washington hops and from 50 to 100 pounds of New York and imported hops were distilled. The California hops were from Perkins, Cosumne, Ukiah, and Wheatland. The Oregon samples were from Independence, the Washington samples from Chehalis, and the New York samples from near Waterville. All were representative samples of commercial, sulphured hops. The imported hops were from Saaz, Bohemia. The distilling apparatus consists of a steel body, steel head, condenser, and receiver.[a] The material to be distilled was packed firmly in the still, to which the steel head was then securely clamped, after which steam was passed slowly through the material and the condensed vapors collected in the receiver, the volatile oil separating in a distinct layer on the aqueous distillate.

The yields of oil obtained from the various hops during the four successive seasons are given in Table I.

TABLE I.—*Yields of volatile oil from various hops during the years 1906 to 1909, inclusive.*

| Source of hops. | Years. | | | | | |
|---|---|---|---|---|---|---|
| | 1906 (cold storage). | 1906 | 1907 | 1908 | 1909 | Average. |
| California: | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Perkins No. 1 | ..... | ..... | 0.20 | 0.38 | 0.43 | 0.336 |
| Cosumne | ..... | ..... | .32 | .24 | .42 | .326 |
| Perkins No. 2 [b] | ..... | ..... | ..... | .37 | .33 | .350 |
| Ukiah | ..... | ..... | .23 | .53 | .28 | .346 |
| Wheatland | ..... | ..... | .21 | .20 | .44 | .283 |
| Oregon | 0.30 | 0.34 | .20 | .32 | .30 | 290 |
| New York | ..... | .32 | .16 | .14 | .15 | .192 |
| Washington | ..... | ..... | ..... | .36 | .38 | .370 |
| Imported (Saaz) | .33 | .45 | .32 | .23 | .24 | .310 |

[a] A detailed description of the distilling apparatus is given in Bulletin 195 of the Bureau of Plant Industry (Rabak, 1910).

[b] Grown at the Brewer ranch, located near Perkins, on the opposite side of the American River.

Considerable variation exists in the yield of oil from the different California hops, not only among the different samples of any one season, but also among the same hops during successive seasons. The average yield of oil from all the California hops during 1907, 1908, and 1909 was 0.32 per cent. Perkins, Cosumne, and Ukiah hops yielded oils which averaged very well during the three seasons. The oil from the Wheatland hops was somewhat below the general average.

The Oregon hops which were distilled during the four successive years showed an average oil content of 0.29 per cent, which is a trifle less than the average California sample.

The New York hops, with an average during the four years of 0.192 per cent of oil, were noticeably lower in oil content than any of the other hops distilled. Only in one year, 1906, did the yield approach that of the California, Oregon, Washington, or the imported hops.

The Washington hops, which were distilled only during two seasons, appear to possess the highest percentage of oil, the average being 0.37 per cent.

The imported hops distilled from the crops of 1906, 1907, 1908, and 1909 showed an average yield of 0.31 per cent of oil, considerable change appearing from season to season.

When distilled for a period of more than two seasons, all the hops showed considerable fluctuation in the yield of oil. Named in the order of their average yield the hops group themselves as follows: Washington, California, imported (Saaz), Oregon, and New York.

This variability in the oil content may be ascribed to varying conditions of climate and soil, as well as to ripeness and drying of the hops, which would affect the formation of the oil in the plant. Slight differences in yield of oil would not necessarily influence the quality, since the same proportion of odoriferous constituents may still be present.

## PHYSICAL PROPERTIES OF THE VARIOUS OILS

The physical properties of the oils permit a somewhat better means for comparison, and therefore a record was made of the color, odor, and taste of each of the oils mentioned. Differences in color are, of course, readily noted, but differences in odor and taste are considerably less noticeable, because of the difficulty with which the senses of smell and taste distinguish closely related substances. The specific gravity, refractive power, and solubility, each of which can be accurately measured, are of much greater importance, although even these properties are usually entirely inadequate for detecting constant differences. However, as they are affected by the constituents of the oils they are sometimes of considerable value. In all cases the oils were too dark to permit making determinations of the optical rotation, which is often useful in detecting certain differences in composition. The tabulation shows the physical properties of the various hop oils distilled during the seasons 1907, 1908, and 1909 (Table II).

TABLE II.—*Physical properties of various hop oils distilled during several successive seasons.*

COLOR.

| Source of hops. | 1907 crop. | 1908 crop. | 1909 crop. |
|---|---|---|---|
| California: | | | |
| Perkins No. 1 | Brownish red | Dark brownish red | Light brownish red. |
| Cosumne | Light brownish red | do | Dark brownish red. |
| Perkins No. 2 | | Pale brown | Wine red. |
| Ukiah | Dark brownish red | Reddish brown | Do. |
| Wheatland | Pale brown | do | Reddish brown. |
| Oregon | Wine red | do | Dark reddish brown. |
| New York | Deep brownish red | Bright brownish red | Deep brownish red. |
| Washington | | Light brownish red | Light wine red. |
| Imported (Saaz) | Brown | Brownish red | Dark brown. |

ODOR.

| | | | |
|---|---|---|---|
| California: | | | |
| Perkins No. 1 | Strongly aromatic, characteristic. | Strongly aromatic, musty, not agreeable. | Strongly aromatic, not unpleasant. |
| Cosumne | Agreeable, hop-like, very aromatic. | Pleasant, aromatic, hop-like. | Strongly aromatic, agreeable. |
| Perkins No. 2 | | Slightly musty, sour, strongly aromatic. | Very pleasant, flowery. |
| Ukiah | Strongly aromatic. | Strongly aromatic. | Slightly fatty, aromatic. |
| Wheatland | Strongly aromatic, disagreeable. | Strongly aromatic, pleasant, characteristic. | Strongly aromatic, not agreeable. |
| Oregon | Extremely strong aromatic, not agreeable. | Strongly aromatic, slightly musty. | Strongly aromatic, pleasant. |
| New York | Strong, characteristic, not agreeable. | Musty and unpleasant, aromatic. | Strongly aromatic, not agreeable. |
| Washington | | Agreeable and strongly aromatic. | Agreeable and aromatic. |
| Imported (Saaz) | Mild, aromatic, agreeable. | Very pleasant, characteristic. | Pleasant, mild, characteristic. |

TASTE.

| | | | |
|---|---|---|---|
| California: | | | |
| Perkins No. 1 | Slightly fatty, slightly pungent. | Slightly pungent, slightly bitter. | Slightly pungent, slightly bitter. |
| Cosumne | Aromatic, not pungent, slightly bitter. | Fatty, slightly bitter, aromatic. | Fatty, bitter, pungent. |
| Perkins No. 2 | | Slightly fatty, aromatic, faintly pungent. | Slightly fatty, bitter, faintly pungent. |
| Ukiah | Bitter, slightly pungent, aromatic. | Fatty, slightly pungent, aromatic. | Fatty, becoming aromatic and bitter. |
| Wheatland | Bitter, aromatic, slightly pungent. | Bitter, aromatic, slightly pungent. | Fatty, slighty bitter, pungent. |
| Oregon | Slightly pungent, becoming slightly bitter. | Fatty, becoming slightly bitter. | Slightly fatty, slightly bitter. |
| New York | Bitter, aromatic, not pungent. | Slightly fatty and bitter, slightly pungent. | Slightly sour, slightly bitter and pungent. |
| Washington | | Slightly fatty and bitter, very little pungency. | Slightly bitter, slightly pungent. |
| Imported (Saaz) | Fatty, slightly bitter, not pungent. | Fatty, aromatic, slightly pungent. | Fatty, aromatic, very slightly pungent. |

TABLE II.—*Physical properties of various hop oils distilled during several successive seasons*—Continued.

SPECIFIC GRAVITY AND INDEX OF REFRACTION.

| Source of hops. | Specific gravity, 20° C. | | | Index of refraction at 20° C. | | |
|---|---|---|---|---|---|---|
| | 1907 crop. | 1908 crop. | 1909 crop. | 1907 crop. | 1908 crop. | 1909 crop. |
| California: | | | | | | |
| Perkins No. 1 | 0.821 | 0.838 | 0.8316 | 1.4838 | 1.4783 | 1.4716 |
| Cosumne | .821 | [a].8395 | .842 | 1.4825 | 1.4724 | 1.4733 |
| Perkins No. 2 | ..... | [a].8289 | .8422 | ..... | 1.4691 | 1.4743 |
| Ukiah | .821 | [a].831 | [a].839 | 1.4890 | 1.4737 | 1.4718 |
| Wheatland | .828 | .8443 | .8358 | 1.4870 | 1.4753 | 1.4743 |
| Oregon | .8343 | .838 | .8433 | 1.4802 | 1.4730 | 1.4705 |
| New York | [b].859 | [b].834 | [b].8747 | 1.4804 | 1.4756 | 1.4800 |
| Washington | ..... | .850 | .8464 | ..... | 1.4763 | 1.4734 |
| Imported (Saaz) | [b].852 | [b].821 | [b].858 | 1.4905 | 1.4852 | 1.4829 |

[a] Specific gravity at 23° C. [b] Specific gravity at 24° C.

SOLUBILITY.[a]

[Quantity of oil dissolved in 3 volumes of 94 per cent alcohol.]

| Source of hops. | 1907 crop. | 1908 crop. | 1909 crop. |
|---|---|---|---|
| California: | | | |
| Perkins No. 1 | 0.55 volume oil, turbid, yellowish residue. | 0.7 volume oil, turbid. | 0.8 volume oil. |
| Cosumne | 0.5 volume oil, whitish residue. | 0.85 volume oil, brownish residue. | Do. |
| Perkins No. 2 | ..... | 0.7 volume oil, turbid. | 0.75 volume oil, slightly turbid. |
| Ukiah | 0.35 volume oil, slight turbidity, yellowish-brown residue. | 0.85 volume oil, turbid. | 0.95 volume oil, slightly turbid, with reddish residue. |
| Wheatland | 0.35 volume oil, turbid, yellowish residue. | 0.75 volume oil, brown residue. | 0.7 volume oil. |
| Oregon | 0.65 volume oil, turbid, yellow residue. | 0.9 volume oil | 1 volume oil. |
| New York | 0.85 volume oil, brown residue. | 0.85 volume oil | 0.8 volume oil, light brown. |
| Washington | ..... | 0.85 volume oil, turbid. | 0.9 volume oil. |
| Imported (Saaz) | 0.75 volume oil, slightly turbid. | 0.5 volume oil, yellow viscous residue. | 0.75 volume oil, slightly turbid, bark-brown, viscous residue. |

[a] Solubility of 1907 crop determined after two years; 1908 crop determined after one year.

Color, odor, and taste, which appeal solely to the senses, are not especially significant. The colors of the various oils, ranging from a golden yellow to a dark brown, were due largely to the condition of the material and the time of the distillation. The first runnings obtained in all of the distillations were nearly colorless, but gradually deepened in color as the distillation progressed. The predominant colors seemed to be red and brown, the golden-yellow color being obtained only when small quantities of hops were distilled on a small laboratory scale. Whenever large quantities (100 to 200 pounds) were distilled, the resulting oil invariably possessed a dark color, the particular tint varying with the season. No constant difference was observed in oils from the various sources.

The odor in all cases was naturally characteristic of hops. In some instances, however, a slightly musty odor was perceptible. The oils from the American hops were all strongly aromatic and in most cases agreeable. A slight yet distinctly musty odor was perceived in several of the oils, due probably to imperfect drying and subsequent sweating of the hops in the bale. The oils from the foreign hops seemed to be distinctly different from the American oils, possessing a very pronounced flowery odor, combined with a fatty odor, the effect being most agreeable.

The sense of taste, which is influenced directly by the sense of smell, is generally capable of distinguishing definite and characteristic qualities of a substance, such as pungency and bitterness. Acidity and fattiness can also be easily detected. All of the oils in question had a decidedly aromatic taste, and there were also a number in which bitterness, fattiness, and acidity were very pronounced. The oils from the California hops were all characterized by bitterness and pungency, with slight fattiness. The oils from the imported hops were strongly fatty with only very slight bitterness and pungency.

The densities of the California oils bore a close relationship during individual seasons, differing somewhat from season to season. This would seem to indicate that the approximate composition during any one season was about the same in the several oils. The average specific gravity of the several California oils for the three seasons was about 0.8326. It will be seen that the Oregon oils were somewhat higher, the average being 0.8385. The oils from the imported hops, with an average specific gravity of 0.8433, and the Washington oils, with an average of 0.8482, followed in order. The oils with the highest general specific gravity were those from the New York hops, which averaged 0.8554 at 24° C. This figure would be increased if corrected to the temperature at which the specific gravity of the majority of the oils was recorded. It is generally acknowledged that the specific gravity is modified by the composition of an oil, but it is doubtful whether the differences noted above would cause any remarkable change in the quality of the oil. A

high specific gravity would usually be accompanied by a larger percentage of high-boiling constituents, and vice versa.

From Table II it is readily seen that the refraction of the California oils during each of the three years shows but little variation. During the successive seasons the refraction of the oils from the 1907 crop was somewhat higher than that of the two following years. This was accounted for by the fact that the index of refraction of the oils of the 1907 crop was taken about two years later (1909), showing that a change had taken place in the oils. The refractive property of the Oregon, New York, and Washington oils, as compared with the California oils, was not greatly different. However, the oils from the imported hops showed a higher refractive index than any of the other oils. This, again, may be due to the presence of a somewhat higher percentage of highly refractive constituents in these oils.

The solubility of a volatile oil in alcohol depends upon the composition of the oil. A high percentage of terpenes and sesquiterpenes decreases the solubility and a high content of oxygenated compounds increases it. Owing to the insolubility of hops oils in alcohol and the difficulty thereby encountered in obtaining comparative results, a deviation was made from the usual method employed for determining solubility. One volume of the oil was thoroughly shaken with three volumes of 94 per cent alcohol in a graduated cylinder, after which the resinous insoluble matter was centrifuged. The amount of insoluble matter could then be easily read on the bottom of the cylinder and the percentage of dissolved material readily calculated.

Apparently the most soluble oil among the number was the oil from the Oregon hops, one volume of oil from the 1909 crop dissolving completely in three volumes of 94 per cent alcohol, the oil from the 1908 crop being almost as soluble. Washington and New York oils from the crops of 1908 and 1909 were slightly less soluble than the Oregon oils. The California oils of these two seasons were a trifle less soluble than those from the Washington and New York hops, while the imported oils appeared to be the least soluble.

The much lower solubility of the 1907 oils was due to the fact that the determinations were not made until two years after distillation. Although the oils had been kept in well-filled bottles and well protected from the light, decomposition had ensued, which resulted in the formation of less soluble constituents, thus decreasing the solubility of the oils. This plainly shows the effect of age on the solubility of the oils.

From the information thus obtained it would appear that the oils with the highest solubility probably contained a larger percentage of oxygenated compounds and a lower percentage of terpenic compounds than the less soluble oils.

## CHEMICAL PROPERTIES OF THE VARIOUS OILS

In order to make a better comparison of the several oils with regard to their aromatic quality, determinations were made of the acid, ester, and saponification numbers. These constants are usually ascertained in order to get some idea of the odoriferous constituents.

The determination of the acid number is readily accomplished by simple titration with standard alkali and is expressed by the number of milligrams of potassium hydroxid required to neutralize the free acidity contained in 1 gram of the oil. A number of factors may tend to influence this value. Freshly distilled oils, in most instances, are low in free acidity, while old oils or oils distilled from old material usually possess a larger quantity of free acids. Improper conditions of drying and storing have a tendency to cause changes to take place in the aromatic compounds, which result in the formation of free acids, and thereby increase the acid numbers.

As previously stated, the esters, which consist of combinations of acids and alcohols, are considered the odor bearers. These values are easily determined by saponifying the oil with alcoholic alkali and calculating the number of milligrams of potassium hydroxid consumed in the reaction by 1 gram of oil. This represents an accurate measurement of the ester constituents. As in the case of free acidity, this value may also be affected by conditions under which the material is dried and stored. The stage of growth and development of the plant is also a strong factor in modifying the ester content of volatile oils.

The saponification number represents the total amount of alkali, expressed in milligrams, necessary to react completely with 1 gram of oil, being the sum of the acid and ester numbers.

These constants which are so directly related to the odor were carefully determined for each of the hop oils distilled from the various samples of hops. A determination of the free alcohols in these oils was also made, but was barren of results in all cases. Acetylization with acetic-acid anhydrid with subsequent saponification is necessary and is readily accomplished in many oils. However, the nature of some alcohols is such that a quantitative acetylization is impossible, owing to decomposition when boiled with the acetic-acid anhydrid. Modification of the usual method, altering the length of boiling and the quantity of acetic anhydrid, gave negative results in all cases. From this peculiar behavior of the oil it is inferred that either the oils have no free alcohol compounds or that the alcohol compounds, if present, are of such a nature as to be incapable of quantitative acetylization.

The acid, ester, and saponification numbers of the oils from the American and foreign hops are recorded in Table III.

TABLE III.—*The acid, ester, and saponification numbers of hop oils distilled during several seasons.*

| Sources of hops. | Acid numbers. | | | | | | Ester numbers. | | | | | | Saponification numbers. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1906 | 1907 | 1908 | 1909 | 1910 | Average. | 1906 | 1907 | 1908 | 1909 | 1910 | Average. | 1906 | 1907 | 1908 | 1909 | 1910 | Average. |
| California: | | | | | | | | | | | | | | | | | | |
| Perkins No. 1 | .... | 0.0 | 1.5 | 1.1 | .... | 0.86 | .... | 42.0 | 47.0 | 47.1 | .... | 45.5 | .... | 42.0 | 48.5 | 48.2 | .... | 46.2 |
| Cosumne | .... | .0 | 2.4 | 2.9 | .... | 1.76 | .... | 45.0 | 46.0 | 51.0 | .... | 47.3 | .... | 45.0 | 48.4 | 53.9 | .... | 49.1 |
| Perkins No. 2 | .... | .... | 1.6 | 2.0 | .... | 1.80 | .... | .... | 45.0 | 43.7 | .... | 44.3 | .... | .... | 46.6 | 45.7 | .... | 46.1 |
| Ukiah | .... | .0 | 1.1 | 1.8 | .... | .96 | .... | 40.8 | 44.0 | 51.0 | .... | 45.2 | .... | 40.8 | 45.1 | 52.8 | .... | 46.2 |
| Wheatland | .... | 2.3 | 2.0 | 1.4 | .... | 1.90 | .... | 50.0 | 45.5 | 41.0 | .... | 45.5 | .... | 52.3 | 47.5 | 42.4 | .... | 47.4 |
| Oregon | 5.5 | 1.6 | 1.0 | 2.8 | .... | 2.70 | 72.0 | 57.0 | 50.2 | 56.0 | .... | 58.8 | 77.5 | 58.6 | 51.2 | 58.8 | .... | 61.5 |
| New York | 4.8 | 3.6 | 2.1 | 2.5 | .... | 3.25 | 44.0 | 61.0 | 47.0 | 51.8 | .... | 50.9 | 48.8 | 64.6 | 49.1 | 54.3 | .... | 54.2 |
| Washington | .... | .... | 1.0 | 1.5 | .... | 1.25 | .... | .... | 51.8 | 53.8 | .... | 52.8 | .... | .... | 52.8 | 55.3 | .... | 54.0 |
| Imported (Saaz) | 1.5 | 1.5 | 1.0 | 3.0 | 3.1 | 2.02 | 12.6 | 20.0 | 36.0 | 28.6 | 20.4 | 23.5 | 14.1 | 21.5 | 37.0 | 31.6 | 23.5 | 21. |

The oils, in the order of their average acidity, were as follows: New York, Oregon, imported, California, and Washington. In every instance the New York oils showed comparatively high acidity. Whether this high acidity content was inherent in the oil or whether it was due to conditions of drying and storing can not be stated. The high acidity seemed to be general, since two other samples of New York hops (not recorded) yielded oils with the acid numbers 2.6 and 3.2, respectively. The average acidity of the Oregon hop oil as given is probably somewhat high, as it was considerably augmented by the high acid number of the oil from the 1906 crop, which was distilled from a sample of hops that were not in as fresh condition as the 1907, 1908, and 1909 hops. The oils from the imported hops were somewhat lower than those from the Oregon hops, being fairly constant in 1906, 1907, and 1908, but much higher in 1909 and 1910. These were followed by the California and Washington oils, with an average of 1.41 and 1.25, respectively.

The high and low acid numbers were significant of nothing important as far as the aroma was concerned, as the free acidity did not perceptibly affect the odor of the oil.

The ester numbers revealed most striking similarities and dissimilarities, not only during one season but for several successive seasons. It was to be expected that the oils from the hops during any one season would show differences, but that these same differences should appear during three, four, and even five successive seasons was most surprising.

The oils from the imported hops were conspicuous because of the fact that the data for the several seasons showed the ester content to be only about one-half as great as the ester content of the oils from the California, Oregon, Washington, and New York hops. Besides the samples recorded in the table, a cold-storage sample of Saaz hops of the 1906 crop, distilled one year later, gave an oil with an ester number of

24. Three samples of Dauber, Auscher, and Oesterreich Gewächs hops of the 1910 crop possessed the ester numbers 15.7, 21.3, and 18, respectively. Figure 1 shows that the nine samples of foreign oils were uniformly lower in esters than the American oils.

The close relationship of the ester numbers of the California oils during the seasons of 1907, 1908, and 1909 is very evident. The general average ester number of the California oils was 45.5, as compared with 50.9 for New York, 52.8 for Washington, 58.8 for Oregon, and 23.5 for the imported. No important difference was noted in the various California oils. This is true also of the Washington oils, which, however, were distilled during only two seasons. The ester content of the Oregon oils averaged considerably higher than the oils from any of the other hops and, with the exception of the oil from the 1906 crop, the history of which was doubtful, the oils bear close relationship from year to year. The New York oils were slightly more variable, but nevertheless occupy about the same relative position from year to year with respect to the other oils.

In the case of the foreign oils, the ester numbers, which are a measure of the odorous constituents, would seem to point to a consistently lower content of these compounds. That this unusually low ester content is responsible for the generally acknowledged superior aroma of imported hops can not be positively stated, although it is highly probable, since it is known that other oils with a low ester content, notably lavender and peppermint oils, are more agreeable and fragrant than oils with a high ester content.

The saponification numbers, which represent the total acids and esters in the oils, presented practically the same constant differences and similarities brought out by comparison of the ester numbers.

While it is not known whether the ester numbers would continue lower indefinitely in case of the foreign oils, it may be assumed that such would probably be the case, since the authentic samples distilled during the five seasons showed abnormally low values as compared with the American oils. In like manner, it may also be assumed that the high ester numbers of the American oils would continue indefinitely, since they were fairly constant during the three seasons in which the experiments were carried on.

## FRACTIONATION OF THE VARIOUS OILS

Since volatile oils are composed of a number of constituents with boiling points which often vary considerably, fractional distillation has proved a useful and effective method for the partial separation of these constituents. Ordinarily a straight-neck distilling flask with side tube is used for this purpose, the bulb of the thermometer being placed immediately opposite the mouth of the tube and the fractions collected at different intervals. By this means a partial separation of the con-

stituents is accomplished. A better separation, however, can be made by means of a 3-bulb Ladenburg distilling flask, which is a flask with

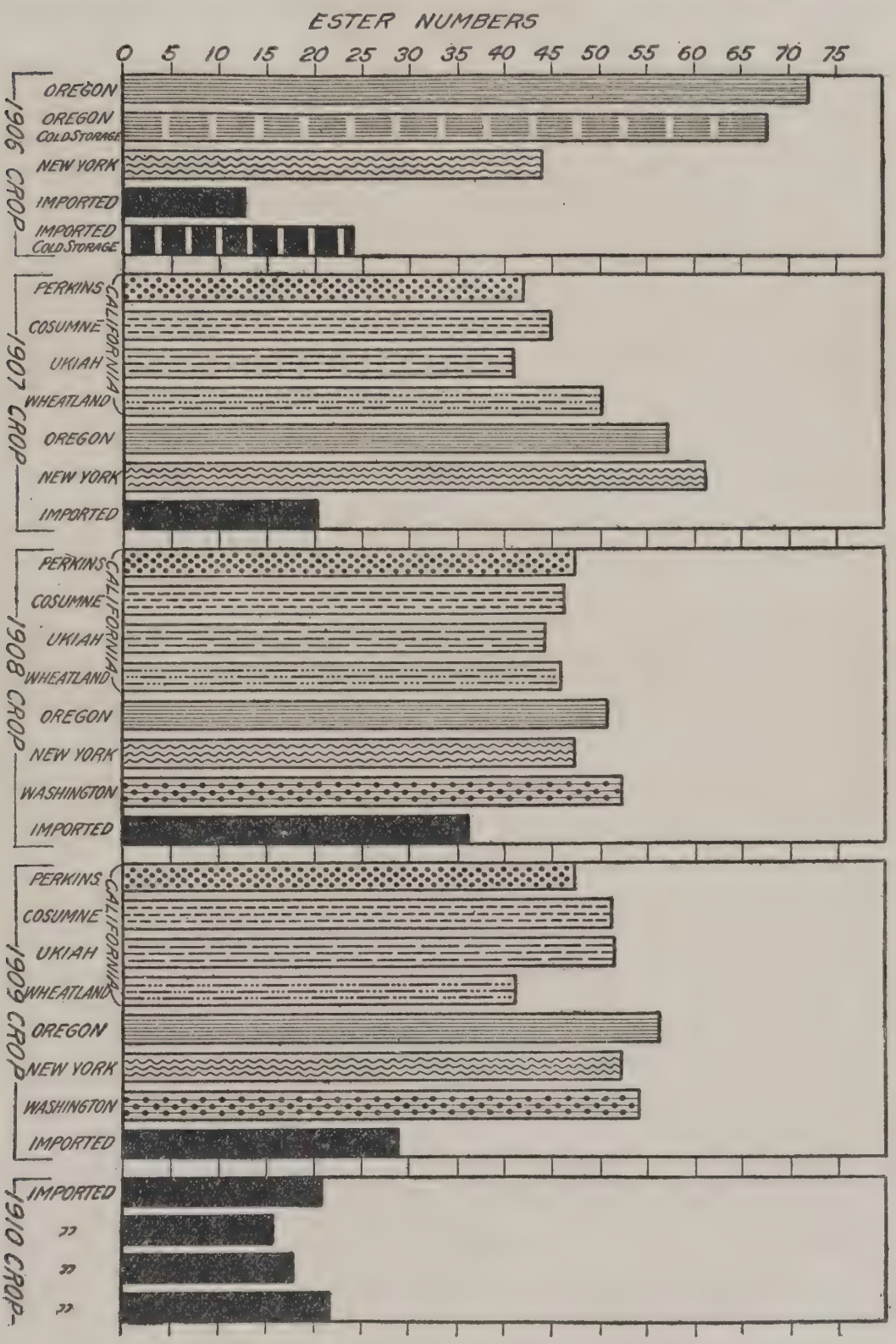



FIG. 1.—Relative ester content of various hop oils.

three bulbs blown in the neck below the side tube and the flask proper. These bulbs act like a distilling column, causing a more complete sepa-

ration of each of the constitutents of the oil. In the fractionation of the various hop oils, a flask of this sort having a capacity of 200 cubic centimeters was employed. The bulb of the thermometer was placed directly opposite the outlet tube in all the experiments. The rapidity of distillation was also regulated so that about the same amount of oil distilled during a given period of time. Fractions were collected between different temperatures, the same range of temperature being maintained in the distillation of each oil. Even with all these precautions it was impossible to make the separation of the compounds entirely complete. Vacuum distillation would possibly have effected the separation with less decomposition, but the difficulty of keeping conditions alike in each case would hardly compensate for the partial decomposition which is unavoidable by direct distillation. With the conditions of distillation practically alike in each fractionation, approximately the same amount of decomposition should result in the high-boiling fractions in each oil.

For purposes of comparison, fractionation by direct distillation was employed with each sample of oil from the various kinds of hops, seven fractions being collected from the oil of the 1906 crop, as follows: Fraction 1, 165° to 185° C.; fraction 2, 185° to 205° C.; fraction 3, 205° to 225° C.; fraction 4, 225° to 245° C.; fraction 5, 245° to 260° C.; fraction 6, 260° to 275° C.; fraction 7, 275° to 290° C.; and finally the residue 290° C. +. Seven fractions were also collected from the oils of the 1907, 1908, and 1909 crops between somewhat different temperatures, as will be seen in the tables. The percentage of oil distilled was calculated for each fraction of the various oils of the crops of 1906, 1907, 1908, and 1909.

In order to facilitate comparisons and to bring out more forcibly the differences in the approximate composition of the oils, as manifested by the fractionation, tabulations were made of the oils distilled each season. The figures in Table IV express the percentage of oil distilled at the temperatures indicated. Curves were also made of each fractionation in order that the comparison could be seen at a glance (see figs. 2, 3, 4, and 5).

TABLE IV.—*Fractionation of hop oils, showing the percentage distilled at different temperatures for the years 1906 to 1909, inclusive.*

1906 CROP.

| Source of hops. | Fraction 1, 165° to 185° C. | Fraction 2, 185° to 205° C. | Fraction 3, 205° to 225° C. | Fraction 4, 225° to 245° C. | Fraction 5, 245° to 260° C. | Fraction 6, 260° to 275° C. | Fraction 7, 275° C.+ | Residue, 290° C.+ |
|---|---|---|---|---|---|---|---|---|
| Oregon..... | 26.9 | 13.1 | 11.55 | 14.7 | 9.2 | 10.3 | 14.25 | ..... |
| Do.[a]..... | 33.5 | 11.4 | 6.4 | 5.7 | 7.9 | 6.4 | 12.4 | 16.0 |
| New York... | 24.5 | 17.5 | 2.5 | 3.5 | 15.2 | 19.2 | 17.6 | ..... |
| Imported (Saaz)... | 9.0 | 9.1 | 2.5 | 8.4 | 25.6 | 27.8 | 17.6 | ..... |
| Do.[a]..... | 10.0 | 9.2 | 8.5 | 10.0 | 30.0 | 18.6 | 5.7 | 8.0 |

[a] In cold storage one year before distillation of the oil.

TABLE IV.—*Fractionation of hop oils, showing the percentage distilled at different temperatures for the years 1906 to 1909, inclusive*—Continued.

1907 CROP.

| Source of hops. | Fraction 1, 165° C. | Fraction 2, 165° C. to 170° C. | Fraction 3, 170° C. to 185° C. | Fraction 4, 185° C. to 225° C. | Fraction 5, 225° C. to 260° C. | Fraction 6, 260° C. to 275° C. | Fraction 7, 275° C. to 290° C. | Residue, 290° C. |
|---|---|---|---|---|---|---|---|---|
| California: | | | | | | | | |
| Perkins | 18.7 | 26.5 | 18.7 | 7.5 | 5.0 | 5.0 | 10.6 | 8.1 |
| Cosumne | 24.3 | 15.0 | 14.3 | 5.0 | 3.1 | 7.5 | 14.3 | 16.5 |
| Ukiah | 22.0 | 29.0 | 9.0 | 6.7 | 4.0 | 3.2 | 11.5 | 14.6 |
| Wheatland | 19.0 | 16.0 | 18.5 | 12.0 | 11.0 | 6.0 | 9.0 | 8.5 |
| Oregon | 5.5 | 14.0 | 25.0 | 14.5 | 13.0 | 6.5 | 9.5 | 12.0 |
| New York | .0 | 5.7 | 15.7 | 18.8 | 22.8 | 11.0 | 12.8 | 13.2 |
| Imported (Saaz) | .0 | 1.7 | 10.0 | 20.5 | 30.0 | 9.1 | 10.7 | 18.0 |

1908 CROP.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| California: | | | | | | | | |
| Perkins | 3.5 | 6.5 | 25.0 | 14.5 | 13.0 | 4.5 | 14.5 | 18.5 |
| Cosumne | 6.5 | 11.5 | 32.0 | 18.0 | 11.6 | 6.0 | 8.0 | 15.8 |
| Ukiah | 6.0 | 16.3 | 23.6 | 14.8 | 7.2 | 8.0 | 8.3 | 15.8 |
| Wheatland | 4.0 | 11.7 | 27.4 | 17.4 | 12.5 | 9.3 | 8.7 | 9.0 |
| Oregon | 7.0 | 7.7 | 23.0 | 20.0 | 17.0 | 8.8 | 6.0 | 8.0 |
| New York | 3.1 | 15.3 | 25.3 | 18 8 | 16.6 | 6.8 | 6.0 | 8.0 |
| Washington | 3.5 | 4.0 | 12.7 | 20.0 | 18.0 | 13.3 | 11.5 | 17.0 |
| Imported (Saaz) | 4.9 | 13.3 | 24.4 | 15.3 | 7.7 | 5.3 | 10.7 | 18.1 |

1909 CROP.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| California: | | | | | | | | |
| Perkins | 2.5 | 7.0 | 15.0 | 10.0 | 7.5 | 15.4 | 23.4 | 19.2 |
| Cosumne | 5.4 | 4.3 | 9.1 | 12.5 | 13.0 | 14.6 | 21.2 | 19.9 |
| Ukiah | 3.5 | 11.5 | 21.5 | 20.0 | 12.0 | 7.5 | 12.0 | 12.0 |
| Wheatland | 4.1 | 5.8 | 20.8 | 15.4 | 6.6 | 5.4 | 20.0 | 21.9 |
| Oregon | 4.1 | 8.3 | 20.8 | 21.4 | 15.8 | 9.1 | 7.5 | 13.0 |
| New York | 5.0 | 6.5 | 11.0 | 8.5 | 18.0 | 11.9 | 13.1 | 26.0 |
| Washington | 3.7 | 7.2 | 19.0 | 21.5 | 16.5 | 8.8 | 10.6 | 12.7 |
| Imported (Saaz) | ..... | 4.2 | 5.4 | 10.6 | 25.0 | 13.2 | 15.2 | 26.4 |

## COMPARISON OF FRACTIONATED OILS

As shown by Table IV and figure 2, the fractionation of the oils from the Oregon cold storage and Oregon and New York hops follow entirely different lines from the imported and the cold-storage imported hops. In case of the first three, fraction 1 (165° to 185° C.) represents the major portion of the oil, while the oils from the imported hops are directly the reverse, fractions 5 (245° to 260° C.) and 6 (260° to 275° C.) representing more than one-half of the oils. These results indicate that the oils from

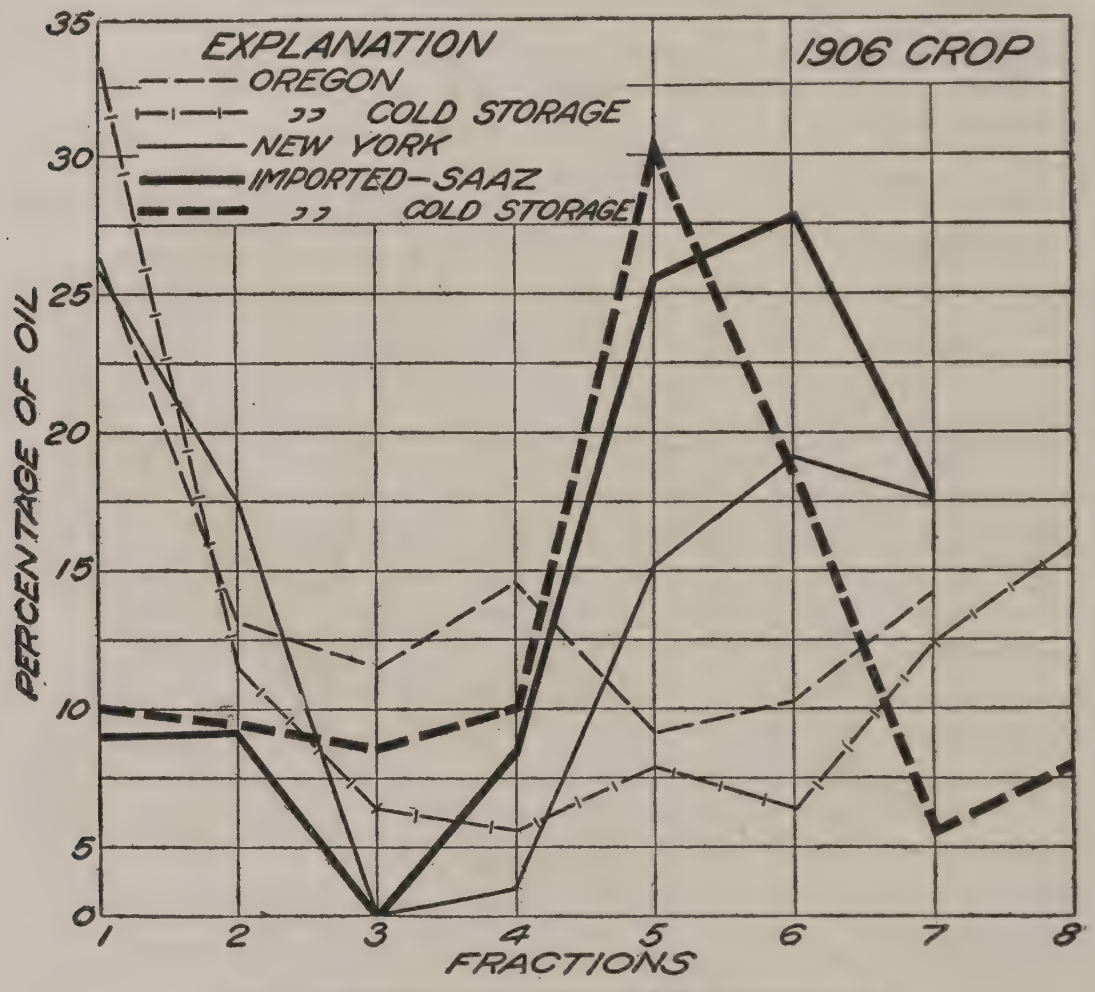



FIG. 2.—Fractionation curves of hop oils from the crops of 1906.

the American hops of the 1906 crop are richest in the low-boiling constituents and the imported oils from the same year are richest in the high-boiling constituents.

The fractionation of the oils of the 1907 crops, as shown also in Table IV and in figure 3, bears out practically the same conclusions. Fractions 1, 2, and 3 (−165°, 165° to 170°, and 170° to 185° C.) of the California oils comprise from 53 to 63 per cent of the original oils, and of the Oregon oil nearly 45 per cent, while the same three fractions of the New York oil correspond to 21 per cent of the original oil. The portion of the imported

oil distilling below 185° C. was only 11.7 per cent of the oil. These results again show the deficiency of the imported oil in the low-boiling constituents. When fractions 4 (185° to 225° C.) and 5 (225° to 260° C.) are considered the reverse conditions exist, these fractions of the imported oils representing about 50 per cent of the oil, 41 per cent of the New York oil, 27 per cent of the Oregon oil, and an average of 13 per cent of the California oils. The curves of the remaining fractions are very similar. The figure shows that the imported oil follows almost a directly reverse course from that of the California and Oregon oils. In this respect the New York oil seems to be the most closely related to the foreign oil.

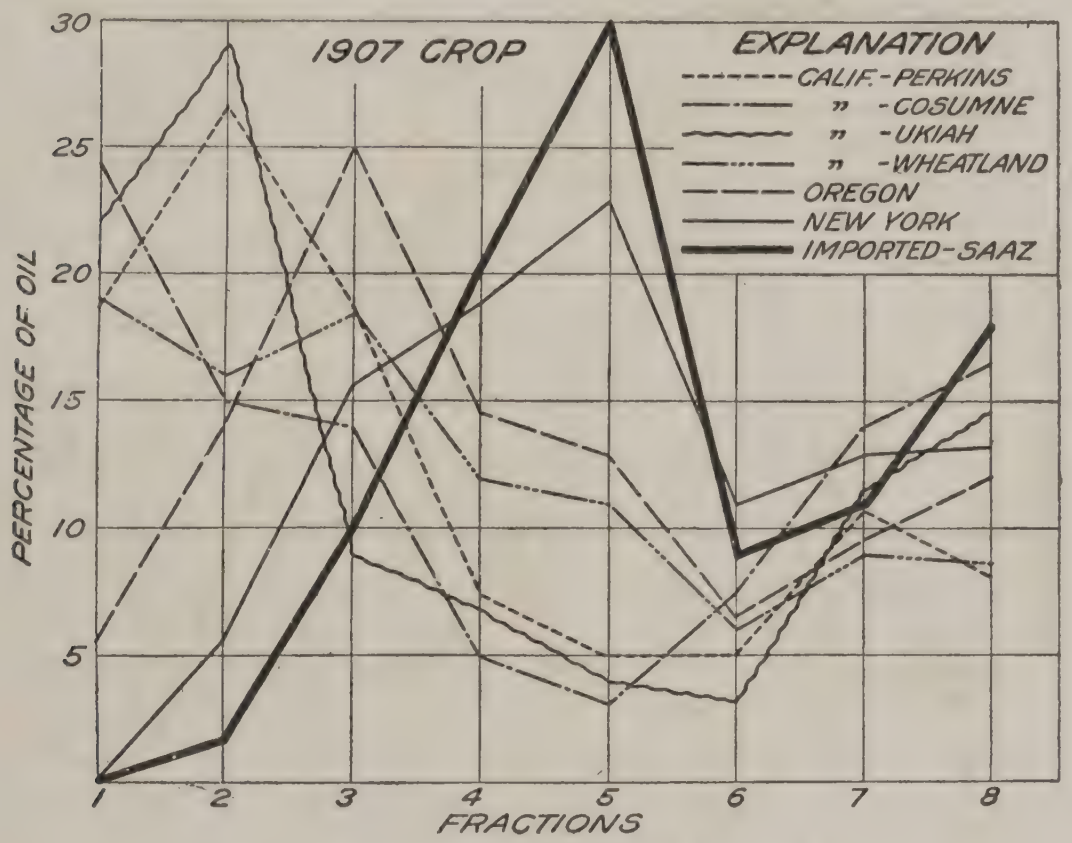



FIG. 3.—Fractionation curves of hop oils from the crops of 1907.

Comparing the various oils of the 1908 crop, as given in Table IV and the curves in figure 4, it will be seen that nearly all show a similar trend in their fractionation properties. The California oils, on an average, again surpass the other oils in constituents which boil below 185° C., the lowest in this respect being those from Washington and Oregon. In that year, for the the first time in the three seasons, the fractionation curve of the imported oil followed lines somewhat similar to those of the American oils. No explanation is ventured for this change in the imported oil.

The difference between the imported oil and the other oils of the 1909 crops is also very noticeable in Table IV and figure 5. The California oils of the 1909 crop, as in 1906, 1907, and 1908, distilled over largely in the

first four fractions, the Cosumne oil showing slight deviation. The curve of the first four fractions of the imported oil is again far below that of any of the other oils. From fraction 4 the New York oil follows a similar course to that of the imported oil. Fraction 5 (225° to 260° C.), in both the imported and the New York oils, shows the highest percentage. The similarity of the Oregon and Washington oils is noteworthy, both curves following almost identical lines.

As a general thing, it will be observed that the oils from the California, Oregon, and Washington hops during the several seasons showed a high

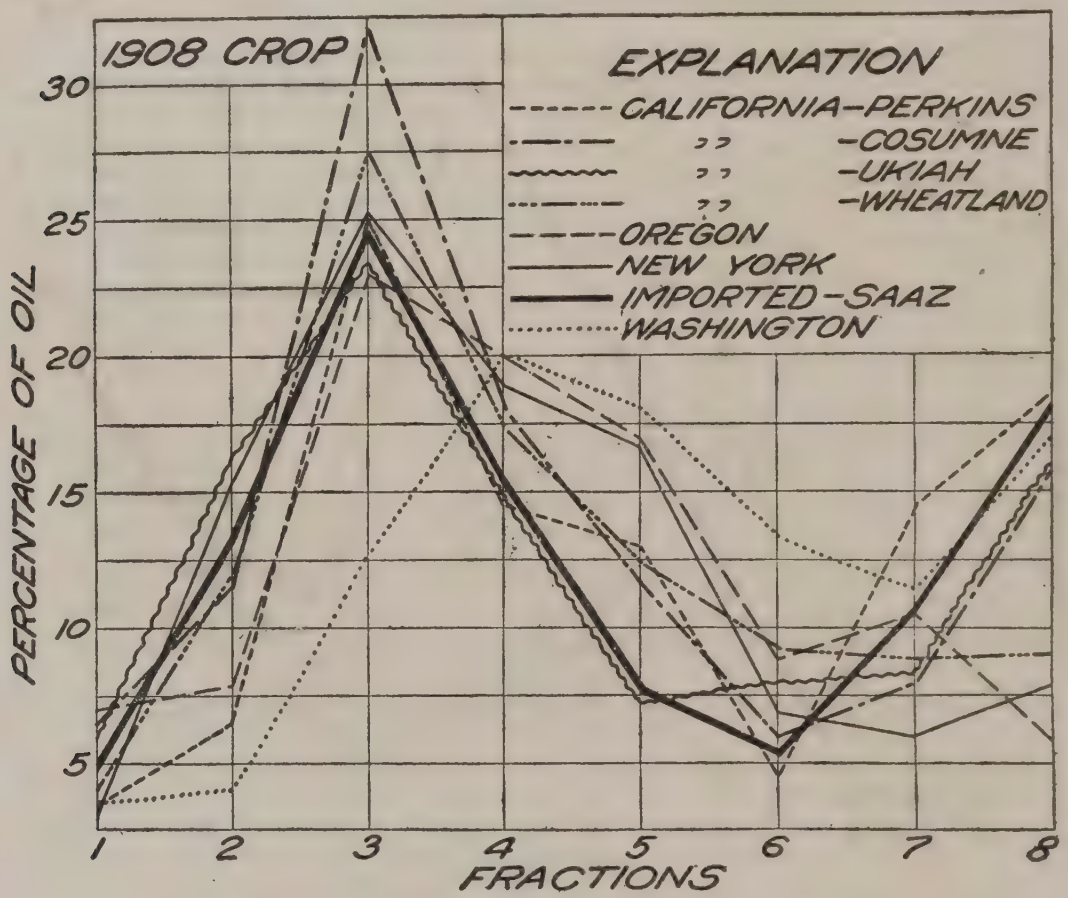



FIG. 4.—Fractionation curves of hop oils from the crops of 1908.

content of low-boiling constituents, while the oils from the imported hops were invariably poorer in the low-boiling constituents. On the other hand, the oils from the imported hops seemed, generally speaking, to contain much higher percentages of the high-boiling compounds, as shown by fractions 4 and 5; the oil of the 1908 crop, however, was unique in that it appeared to be similar to the other oils during that particular season. The curves of the imported oils followed those of the New York oils the most closely, the general direction being similar. The California oils also followed very similar directions, as did the Oregon and Washington oils.

## PHYSICAL PROPERTIES OF THE FRACTIONS

### SPECIFIC GRAVITY

Although the specific gravity, as a rule, is apt to show only slight differences, owing to the variable composition of the oil, it was thought that determinations of this property might be of some value. The specific gravity of the various fractions from the oils of the crops of 1906, 1907, 1908, and 1909 is given in Table V.

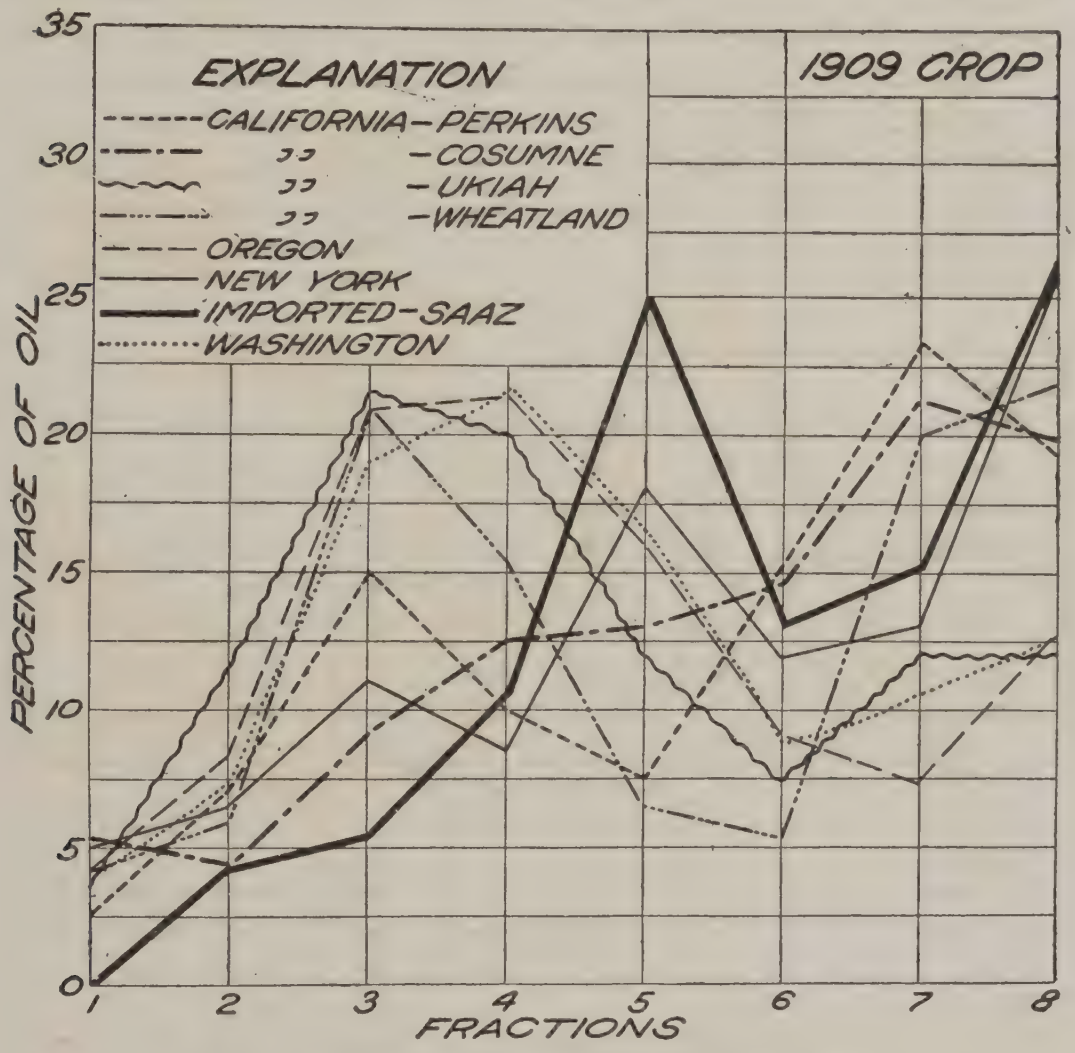



FIG. 5.—Fractionation curves of hop oils from the crops of 1909.

TABLE V.—*Specific gravity of fractions of hop oils for the years 1906 to 1909, inclusive.*

1906 CROP (AT 25° C.).

| Source of hops. | Fraction 1, 165° to 185° C. | Fraction 2, 185° to 205° C. | Fraction 3, 205° to 225° C. | Fraction 4, 225° to 245° C. | Fraction 5, 245° to 260° C. | Fraction 6, 260° to 275° C. | Fraction 7, 275° C. |
|---|---|---|---|---|---|---|---|
| Oregon | 0.818 | 0.821 | 0.849 | 0.876 | 0.877 | 0.881 | ..... |
| Do.[a] | .820 | .839 | .859 | .882 | .894 | .900 | 0.887 |
| New York | .813 | .829 | .853 | .862 | .890 | .894 | ..... |
| Imported | .815 | .819 | .825 | .835 | .888 | .888 | ..... |
| Do.[a] | .826 | .838 | .850 | .873 | .891 | .896 | .836 |

[a] In cold storage one year before distillation.

TABLE V.—*Specific gravity of fractions of hop oils for the years 1906 to 1909, inclusive*—Continued.

1907 CROP (AT 25° C.).

| Source of hops. | Fraction 1, —165° C. | Fraction 2, 165° C. to 170° C. | Fraction 3, 170° C. to 185° C. | Fraction 4, 185° C. to 225° C. | Fraction 5, 225° C. to 260° C. | Fraction 6, 260° C. to 275° C. | Fraction 7, 275° C. to 290° C |
|---|---|---|---|---|---|---|---|
| California: | | | | | | | |
| Perkins | 0.803 | 0.806 | 0.815 | 0.843 | 0.875 | 0.889 | 0.876 |
| Cosumne | .809 | .811 | .819 | .840 | .875 | .871 | .862 |
| Ukiah | .806 | .810 | .824 | .848 | .881 | .891 | .881 |
| Wheatland | .809 | .810 | .816 | .845 | .893 | .900 | .895 |
| Oregon | .809 | .811 | .816 | .855 | .889 | .895 | .876 |
| New York | ..... | .812 | .821 | .853 | .884 | .891 | .876 |
| Imported (Saaz) | ..... | ..... | .812 | .832 | .874 | .887 | .878 |

1908 CROP (AT 23° C.).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| California: | | | | | | | |
| Perkins | 0.802 | 0.805 | 0.809 | 0.834 | 0.883 | 0.871 | 0.866 |
| Cosumne | .805 | .804 | .810 | .835 | .884 | .894 | .880 |
| Ukiah | .803 | .804 | .808 | .835 | .882 | .888 | .863 |
| Wheatland | .805 | .806 | .811 | .837 | .885 | .894 | .882 |
| Oregon | .802 | .801 | .806 | .828 | .885 | .890 | .883 |
| New York | ..... | .810 | .813 | .842 | .889 | .897 | .897 |
| Washington | .803 | .805 | .809 | .835 | .881 | .887 | .874 |
| Imported (Saaz) | ..... | .811 | .813 | .840 | .885 | .890 | .876 |

1909 CROP (AT 23° C.).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| California: | | | | | | | |
| Perkins | 0.812 | 0.815 | 0.821 | 0.844 | 0.879 | 0.863 | 0.876 |
| Cosumne | .820 | .822 | .832 | .855 | .891 | .888 | .875 |
| Ukiah | .806 | .807 | .810 | .833 | .873 | .891 | .881 |
| Wheatland | .803 | .807 | .812 | .831 | .872 | .872 | .888 |
| Oregon | .806 | .806 | .811 | .831 | .880 | .894 | .889 |
| New York | .823 | .819 | .828 | .859 | .890 | .900 | .886 |
| Washington | .808 | .808 | .813 | .836 | .884 | .895 | .883 |
| Imported (Saaz) | ..... | .810 | .816 | .837 | .876 | .882 | .872 |

The specific gravity, although less inclined to indicate material differences, at least conveys some idea of the composition of the succeeding fractions. The low-boiling fractions naturally possess the lowest specific gravity, which increases as the constituents of greater density make their appearance in the later fractions. When the specific gravity is fairly constant the fractions may contain similar constituents in similar proportions. A rapid rise during distillation signifies a quick change and a sharp separation of the denser compounds. Sudden increases in specific

gravity from fraction to fraction, as observed in the table, may be construed to mean that a fairly good separation of the denser compounds has taken place. The fractions of the lowest specific gravity in all cases were those boiling below 185° C. This portion of the oil should contain the terpenic constituents if present. Fractions 1, 2, and 3 did not differ greatly from each other through the four seasons, thus indicating a similar composition of the oils. The specific gravity of the oils from the cold-storage samples was greatly different from that of the other oils, being higher in all cases than in the oils from the fresh hops. The specific gravity of fractions 4, 5, and 6 increased very much in the sequence in which they were distilled, comparing very favorably, however, in the different oils. The oxygenated constituents, if present in the oil, would possibly be found largely in fractions 4 and 5, while fractions 6 and 7 should contain sesquiterpene constituents. Fraction 7 showed a decrease in specific gravity, due probably to partial decomposition at the high temperature at which it was distilled.

Curves of the specific gravity were not drawn because of the similarity in the various fractions, the same general course being evident in each oil. In general, the table of results shows that the fractionation of each oil proceeded about the same with regard to the nature of the constituents which distilled over. Although the specific gravities corresponded very closely, it is not necessarily inferred that the oils are alike in composition. The difference may be quantitative rather than qualitative, the size of the fractions determining the quantitative composition of the oils.

## OPTICAL ROTATION

The value of the property of optical rotation, though important in most volatile oils, is perhaps lessened when applied to oil of hops, since its constituents are more or less inactive or only slightly active. The rotatory power of each fraction of the oils was carefully determined, and as it was low it was expressed in minutes rather than degrees. The results were tabulated for each season (Table VI) and curves were drawn to facilitate the comparison (figs. 6, 7, 8, and 9).

TABLE VI.—*Specific rotation of fractions of hop oils distilled for the years 1906 to 1909, inclusive.*

1906 CROP.

| Source of hops. | Fraction 1, 165° to 185° C. | Fraction 2, 185° to 205° C. | Fraction 3, 205° to 225° C. | Fraction 4, 225° to 245° C. | Fraction 5, 245° to 260° C. | Fraction 6, 260° to 275° C. | Fraction 7, 275° C. |
|---|---|---|---|---|---|---|---|
| | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* |
| Oregon | − 7.6 | −20.7 | −15.9 | + 9.4 | +47.0 | +89.2 | ..... |
| Do.[a] | Inactive | −12.3 | −25.1 | −44.6 | +27.8 | +49.9 | +114.5 |
| New York | −11.7 | −15.8 | +42.5 | +60.1 | +34.9 | +52.5 | ..... |
| Imported | Inactive | Inactive | +60.0 | +36.6 | +63.0 | +35.9 | ..... |
| Do.[a] | Inactive | − 4.9 | −11.1 | −14.2 | +11.6 | +67.5 | +177.7 |

[a] In cold storage 1 year before distillation.

TABLE VI.—*Specific rotation of fractions of hop oils distilled for the years 1906 to 1909, inclusive*—Continued.

1907 CROP.

| Source of hops. | Fraction 1, −165° C. | Fraction 2, 165° to 170° C. | Fraction 3, 170° to 185° C. | Fraction 4, 185° to 225° C. | Fraction 5, 225° to 260° C. | Fraction 6, 260° to 275° C. | Fraction 7, 275° to 290° C. |
|---|---|---|---|---|---|---|---|
| California: | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* | *Minutes.* |
| Perkins | −15.0 | −17.7 | −27.3 | −56.3 | −68.8 | −87.1 | − 35.9 |
| Cosumne | −12.7 | −23.6 | −35.2 | −43.2 | −54.5 | +33.2 | + 38.4 |
| Ukiah | −12.8 | −22.7 | −35.2 | −66.0 | −70.8 | −37.3 | + 47.1 |
| Wheatland | −12.7 | −24.7 | −30.4 | −29.4 | −58.7 | −69.1 | +139.0 |
| Oregon | − 3.8 | − 5.7 | −16.9 | −38.3 | −63.0 | +25.5 | + 58.7 |
| New York | ..... | ..... | − 5.8 | − 9.4 | +10.5 | +59.7 | + 93.1 |
| Imported (Saaz) | ..... | ..... | Inactive | − 4.6 | + 1.7 | + 9.2 | + 66.9 |

1908 CROP.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| California: | | | | | | | |
| Perkins | Inactive | Inactive | −20.5 | −44.6 | −66.5 | + 28.4 | +28.4 |
| Cosumne | −25.5 | −25.6 | −28.8 | −44.5 | −72.6 | − 40.0 | +23.4 |
| Ukiah | Inactive | − 6.2 | −10.6 | −39.5 | −58.7 | + 14.7 | ..... |
| Wheatland | −25.5 | −30.7 | −38.2 | −64.2 | −84.3 | − 25.5 | +28.1 |
| Oregon | Inactive | − 5.0 | −16.1 | −26.0 | −13.1 | + 60.4 | + 9.5 |
| New York | ..... | −17.7 | −25.5 | −27.0 | +38.7 | + 88.5 | +76.9 |
| Washington | ..... | Inactive | −18.7 | −27.3 | +37.4 | + 79.3 | +71.1 |
| Imported (Saaz) | Inactive | −15.2 | −25.3 | −44.7 | +77.9 | +104.7 | +85.1 |

1909 CROP.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| California: | | | | | | | |
| Perkins | Inactive | − 9.5 | −24.8 | −51.1 | −66.9 | 4.8 | 23.9 |
| Cosumne | −20.5 | −24.8 | −38.9 | −63.0 | −52.5 | 36.4 | 45.7 |
| Ukiah | ..... | −13.8 | −20.2 | −39.6 | −75.3 | −41.6 | 21.1 |
| Wheatland | −10.2 | −14.3 | −30.5 | −54.8 | −95.2 | −70.0 | 9.6 |
| Oregon | Inactive | Inactive | −10.6 | −16.8 | −14.0 | 83.4 | 107.2 |
| New York | ..... | −15.1 | −21.9 | −29.3 | −11.4 | 36.6 | 65.4 |
| Washington | ..... | −14.8 | −14.8 | −32.5 | 8.3 | 99.6 | 104.1 |
| Imported (Saaz) | ..... | ..... | ..... | −17.1 | −37.6 | −13.9 | 47.4 |

Table VI and figures 6, 7, 8, and 9 show that the initial fractions, as a rule, were inactive. This is probably explained by the fact that these fractions consist of terpenes having little or no activity. The rotation of the first fractions was levo, the power to rotate to the left increasing in most cases up to the fifth fraction. This was par-

ticularly true of the California and Oregon oils of the crops of 1907, 1908, and 1909. The New York, Washington, and imported oils had a general tendency to manifest dextro rotation after the fourth fraction, the levo rotation of the former fractions being consistently less than the same fractions of the California and Oregon oils. The dextro rotation of the last three fractions seemed to be higher, in practically all cases, than

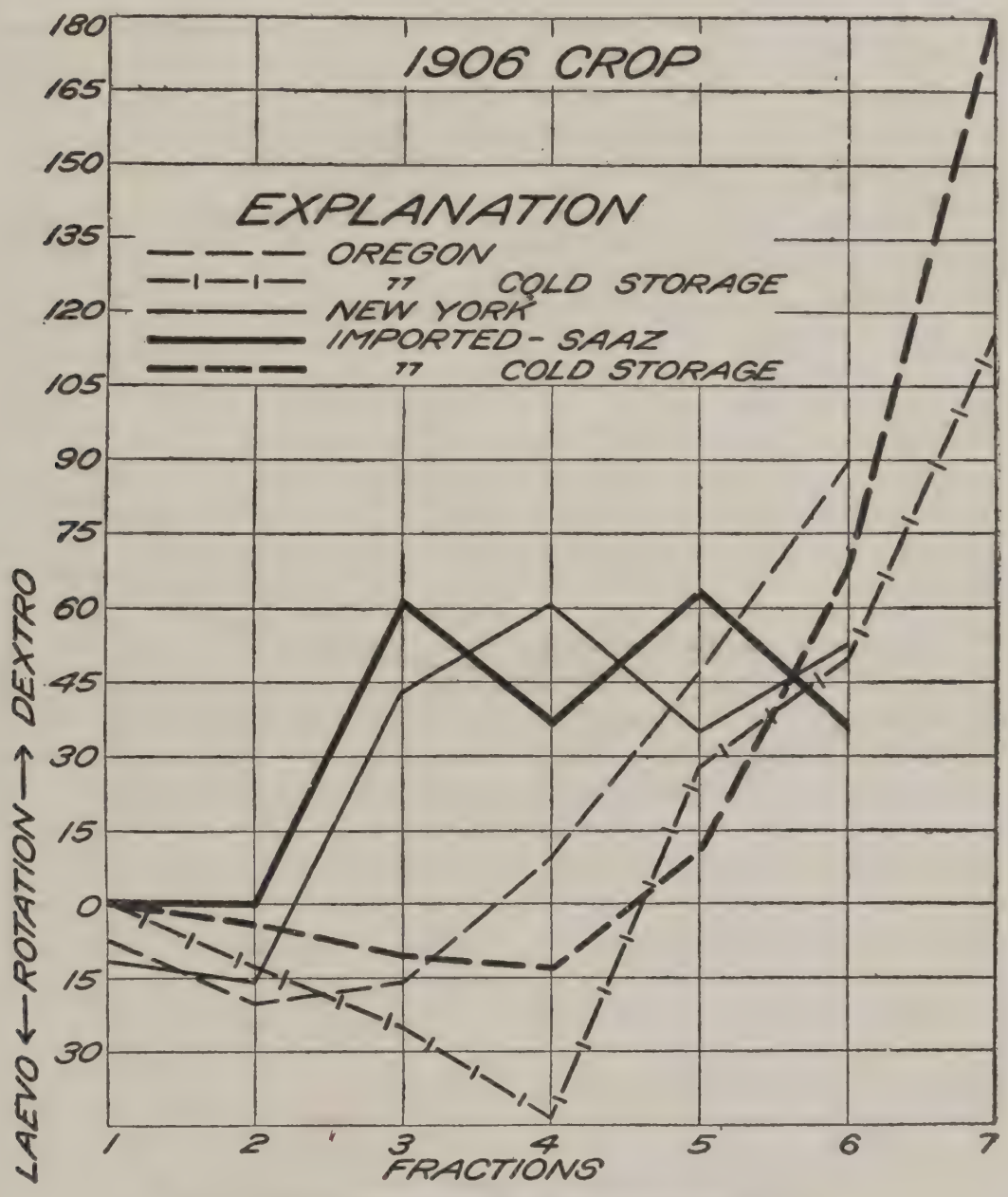



FIG. 6.—Optical-rotation curves of hop oils from the crops of 1906.

the same fractions of California oils. A most noticeable feature was the tendency of the New York oil to follow the same course as the imported oil during each season. The high-boiling portions of the oils, of which the esters and sesquiterpenes form a part, tended to show the greatest rotation. The curves of rotation, as well as the curves of fractionation, show some differences in the various oils, though perhaps in

a lesser degree. This difference appears to be fairly constant from season to season. The same general direction of the California oils during the three seasons is most striking. The curves of the oils of the

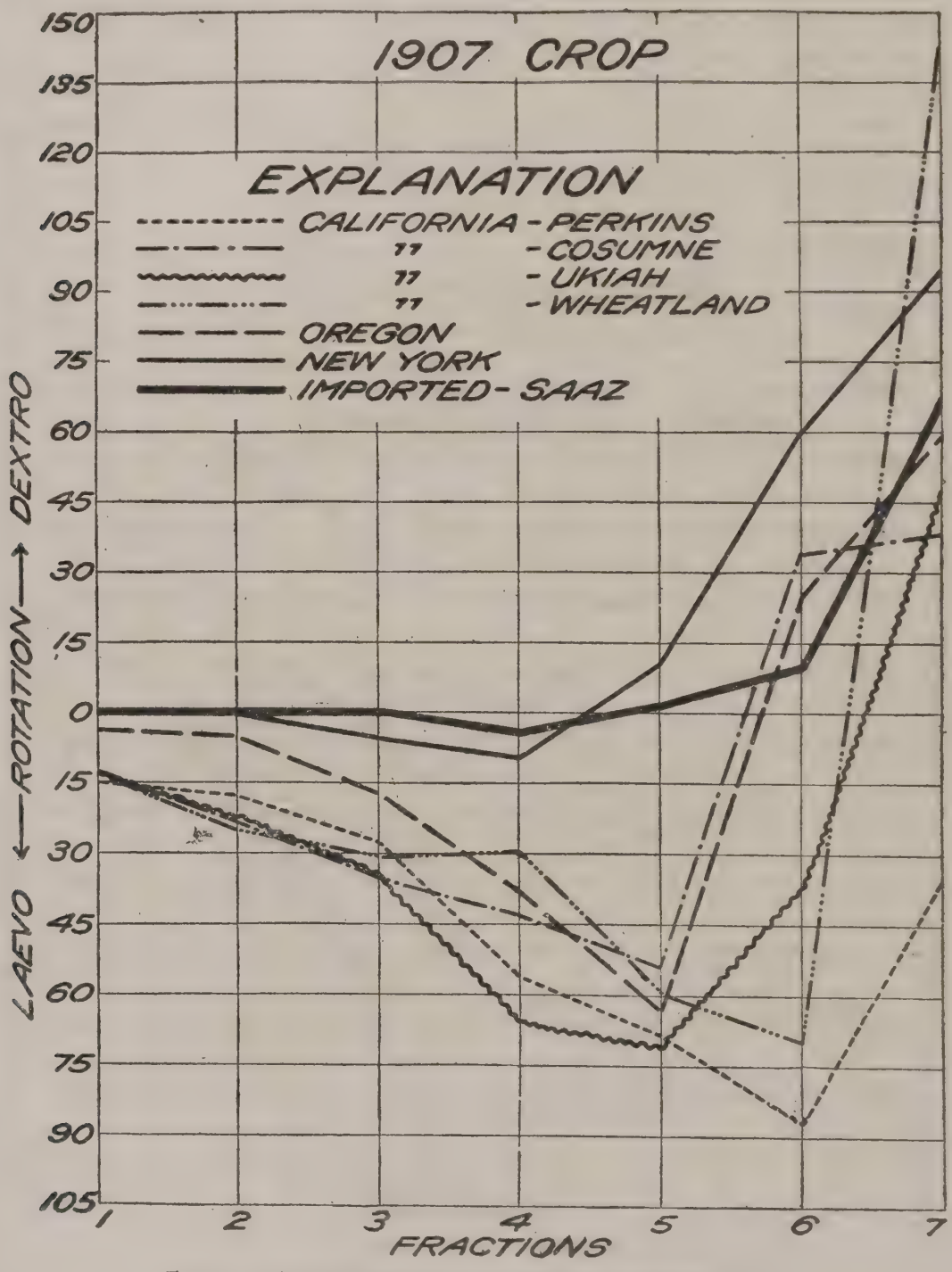



FIG. 7.—Optical-rotation curves of hop oils from the crops of 1907.

1906 crop further emphasize the strong dextro rotation of the oils from the foreign and New York hops as compared with the California and Oregon oils.

## CHEMICAL PROPERTIES OF THE FRACTIONS

### ACID, ESTER, AND SAPONIFICATION NUMBERS

The chemical properties of the oils and fractions are much more important in determining constant differences or similarities in the volatile oil of hops than the physical properties previously discussed. This is especially true of the aromatic quality of the oil, since quality is dependent upon the ester content. The ester content is expressed in the form

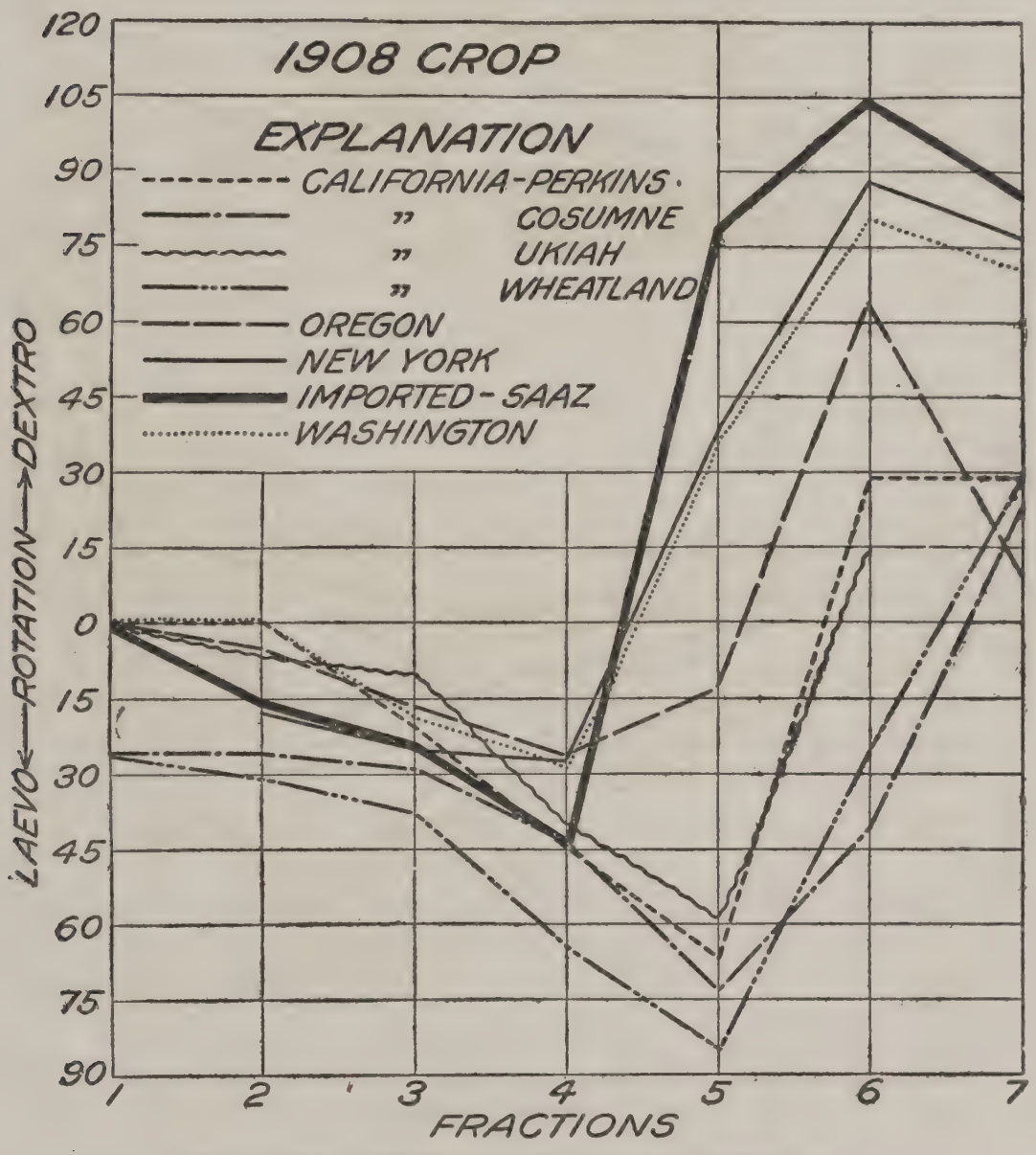



FIG. 8.—Optical-rotation curves of hop oils from the crops of 1908.

of ester numbers based on the amount of esters in 1 gram of the oil. The acid numbers of the fractions are less important, since these depend largely upon the extent of decomposition which the esters undergo during fractionation. Only a small amount of decomposition of the esters is required to liberate sufficient acid to produce high acid numbers, since, as has been shown, the acids in combination with the esters are those of high molecular weight. Owing to the consequent irregularity of the

acid numbers, only little importance can be attached to the results. The saponification numbers, which represent the total saponifiable constituents, including both free acids and esters, should exhibit practically no deviation from the course of the ester numbers. Particular stress is

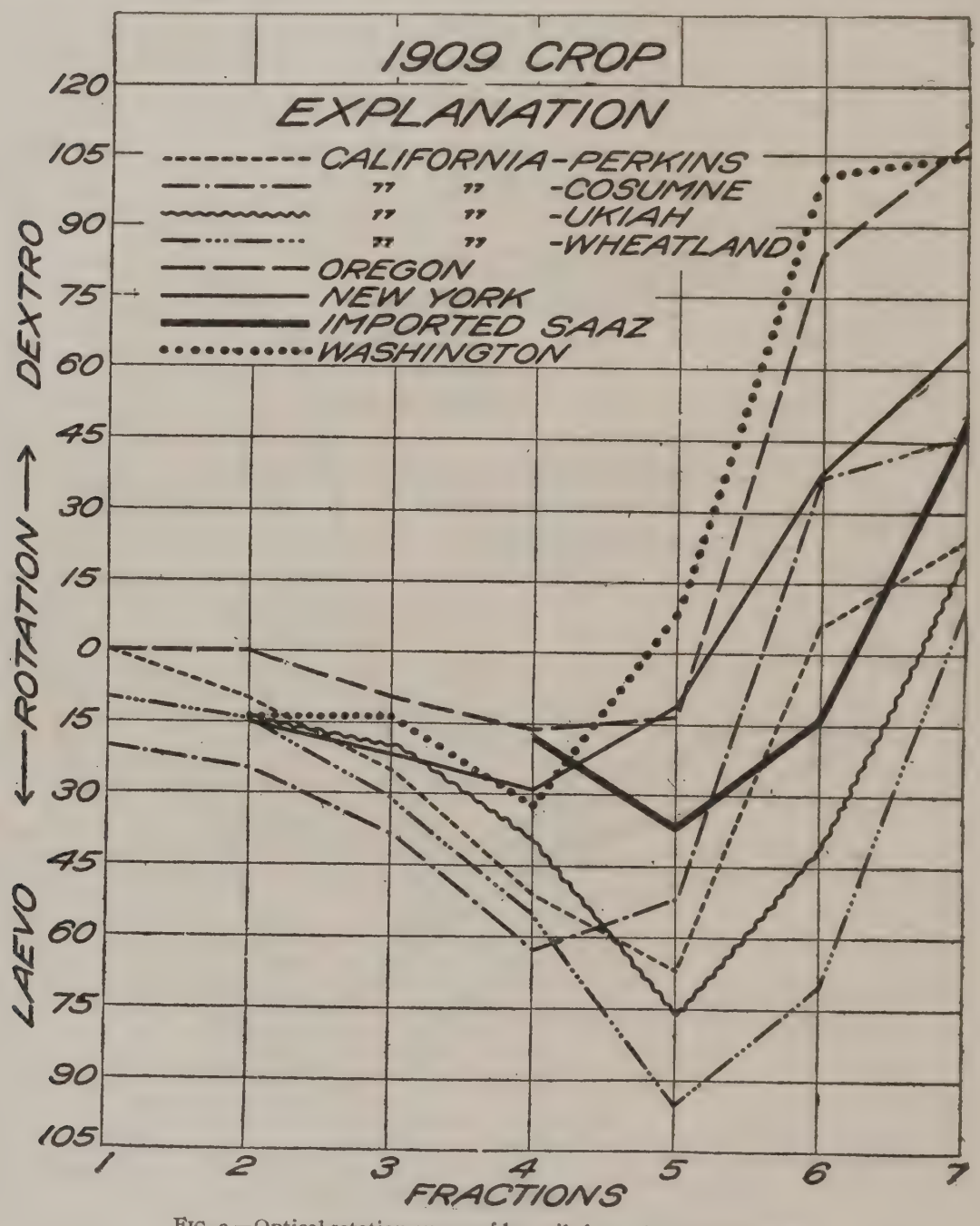



FIG. 9.—Optical-rotation curves of hop oils from the crops of 1909.

therefore placed upon the ester number of the fractions, since these values represent a measurement of the chief odorous constituents of the oils.

The acid, ester, and saponification numbers of the fractions from each individual oil of the seasons of 1906, 1907, 1908, and 1909 are shown in Table VII. Since particular attention is given to the ester numbers, curves were made of this chemical property of the fractions, as shown in figures 10, 11, 12, and 13.

TABLE VII.—*Acid, ester, and saponification numbers of the various fractionated oils from hops for the years 1906 to 1909, inclusive.*

1906 CROP.

| Source of hops. | Fraction 1, 165° to 185° C. | | | Fraction 2, 185° to 205° C. | | | Fraction 3, 205° to 225° C. | | | Fraction 4, 225° to 245° C. | | | Fraction 5, 245° to 260° C. | | | Fraction 6, 260° to 275° C. | | | Fraction 7, 275° C. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. |
| Oregon | 0.0 | 143 0 | 143.0 | 6.9 | 94.0 | 100.9 | 8.0 | 104.0 | 112.0 | 10.8 | 79.0 | 89.8 | 6.6 | 53.3 | 59.9 | 7.7 | 23.0 | 30.7 | 8.2 | 0.0 | 8.2 |
| Do.[a] | 2.8 | 100.0 | 102.8 | 3.6 | 102.0 | 105.6 | 5.5 | 106.3 | 111.8 | 8.1 | 90.5 | 98.6 | 9.2 | 66.6 | 75.8 | 10.5 | 36.3 | 46.8 | 9.0 | 9.6 | 18.6 |
| New York | .0 | 84.4 | 84.4 | 3.7 | 70.0 | 73.7 | 14.0 | 79.0 | 93.0 | 8.2 | 41.0 | 49.2 | 7.9 | 23.0 | 30.9 | 5.1 | 14.3 | 19.4 | 7.4 | .0 | 7.4 |
| Imported | 3.8 | 39.0 | 42.8 | 6.9 | 31.8 | 38.7 | 7.9 | 79.0 | 86.9 | 5.3 | 29.3 | 34.6 | 2.5 | 7.6 | 10.1 | 2.1 | .0 | 2.1 | .0 | .0 | .0 |
| Do.[a] | 4.9 | 60.0 | 64.9 | 4.6 | 50.7 | 55.3 | 2.8 | 52.5 | 55.3 | 4.0 | 44.8 | 48.8 | 3.8 | 16.0 | 19.8 | 3.0 | 6.7 | 9.7 | 9.0 | 3.0 | 12.0 |

1907 CROP.

| Source of hops. | Fraction 1, —165° C. | | | Fraction 2, 165° to 170° C. | | | Fraction 3, 170° to 185° C. | | | Fraction 4, 185° to 225° C. | | | Fraction 5, 225° to 260° C. | | | Fraction 6, 260° to 275° C. | | | Fraction 7, 275° to 290° C. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. |
| California: | | | | | | | | | | | | | | | | | | | | | |
| Perkins | 0.0 | 42.0 | 42.0 | 0.0 | 48.0 | 48.0 | 0.0 | 53.0 | 53.0 | 1.9 | 94.0 | 95.9 | 1.5 | 68.0 | 69.5 | 3.0 | 40.0 | 43.0 | 1.8 | 11.0 | 12.8 |
| Cosumne | .0 | 61.0 | 61.0 | .0 | 65.0 | 65.0 | .0 | 69.0 | 69.0 | 1.7 | 89.0 | 90.7 | .0 | 64.0 | 64.0 | 1.8 | 21.0 | 22.8 | .0 | 3.5 | 3.5 |
| Ukiah | .0 | 44.0 | 44.0 | .0 | 46.5 | 46.5 | .0 | 61.0 | 61.0 | .0 | 70.0 | 70.0 | 3.0 | 58.0 | 61.0 | 5.1 | 38.0 | 43.1 | 2.7 | 10.6 | 13.3 |
| Wheatland | 2.5 | 53.5 | 56.0 | 1.3 | 54.0 | 55.3 | 1.9 | 58.5 | 60.4 | 2.9 | 69.2 | 72.1 | 4.8 | 48.0 | 52.8 | 6.6 | 18.0 | 24.6 | 7.7 | 5.0 | 12.7 |
| Oregon | 2.4 | 62.7 | 65.1 | 1.6 | 65.0 | 66.6 | 1.3 | 68.8 | 70.1 | 1.9 | 91.7 | 103.6 | 2.9 | 75.6 | 78.5 | 4.7 | 27.4 | 32.1 | 4.3 | 10.3 | 14.6 |
| New York | | | | 2.1 | 72.9 | 75.0 | 2.6 | 79.6 | 82.2 | 3.7 | 102.4 | 106.1 | 7.0 | 98.0 | 105.0 | 6.9 | 39.1 | 46.0 | 9.8 | 14.1 | 23.9 |
| Imported (Saaz) | | | | 2.5 | 23.0 | 25.5 | 3.6 | 23.7 | 27.3 | 1.5 | 33.7 | 35.2 | 1.9 | 30.8 | 32.7 | 2.5 | 13.4 | 15.9 | 3.5 | 7.3 | 10.8 |

[a] Hops in cold storage for one year.

41217°—14——5

TABLE VII.—*Acid, ester, and saponification numbers of the various fractionated oils from hops for the years 1906 to 1909, inclusive*—Continued.

1908 CROP.

| Source of hops. | Fraction 1, −165° C. | | | Fraction 2, 165° to 170° C. | | | Fraction 3, 170° to 185° C. | | | Fraction 4, 185° to 225° C. | | | Fraction 5, 225° to 260° C. | | | Fraction 6, 260° to 275° C. | | | Fraction 7, 275° to 290° C. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. | Acid No. | Ester No. | Sap. No. |
| California: | | | | | | | | | | | | | | | | | | | | | |
| Perkins | 2.4 | 53.4 | 55.8 | 1.7 | 56.8 | 58.5 | 1.0 | 63.7 | 64.7 | 2.8 | 86.6 | 89.4 | 4.7 | 71.6 | 76.3 | 3.6 | 26.0 | 29.6 | 2.4 | 8.0 | 10.4 |
| Cosumne | 2.4 | 40.0 | 42.4 | 2.1 | 46.0 | 48.1 | 1.3 | 50.0 | 51.3 | 3.0 | 60.0 | 63.0 | 4.9 | 60.0 | 64.9 | 4.2 | 51.2 | 55.4 | 4.5 | 3.8 | 8.3 |
| Ukiah | 1.0 | 46.0 | 47.0 | .5 | 58.0 | 58.5 | .8 | 58.0 | 58.8 | 1.1 | 78.6 | 79.7 | 2.9 | 71.0 | 73.9 | 2.4 | 23.0 | 25.4 | 2.3 | 3.8 | 6.1 |
| Wheatland | 5.5 | 43.0 | 48.5 | 1.4 | 50.0 | 51.4 | 2.0 | 53.5 | 55.5 | 3.2 | 62.5 | 65.5 | 5.2 | 55.7 | 60.9 | 4.1 | 21.4 | 25.5 | 3.8 | 7.3 | 11.1 |
| Oregon | .7 | 46.3 | 47.0 | .7 | 48.3 | 49.0 | .9 | 53.2 | 54.1 | 1.1 | 75.4 | 76.5 | 2.4 | 69.4 | 71.8 | 2.1 | 21.3 | 23.4 | 3.4 | 8.0 | 11.4 |
| New York | 2.1 | 41.0 | 43.1 | 1.6 | 42.0 | 43.6 | 1.0 | 49.0 | 50.0 | 2.1 | 47.0 | 49.1 | 4.0 | 64.0 | 68.0 | 3.5 | 24.0 | 27.5 | 4.0 | 10.0 | 14.0 |
| Washington | 1.9 | 50.3 | 52.2 | 1.7 | 56.0 | 57.7 | 1.0 | 67.5 | 68.5 | 1.6 | 92.0 | 93.6 | 2.8 | 82.0 | 84.8 | 2.5 | 27.8 | 30.3 | 3.7 | 7.0 | 10.7 |
| Imported (Saaz) | 3.6 | 34.0 | 37.6 | 1.7 | 41.4 | 43.1 | 1.1 | 45.7 | 46.8 | 1.6 | 58.6 | 60.2 | 4.0 | 60.8 | 64.8 | 4.0 | 28.0 | 32.0 | 2.3 | 5.4 | 7.7 |

1909 CROP.

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| California: | | | | | | | | | | | | | | | | | | | | | |
| Perkins | 7.4 | 77.0 | 84.4 | 3.0 | 80.0 | 83.0 | 1.6 | 96.0 | 97.6 | 2.0 | 101.0 | 103.0 | 3.2 | 103.0 | 106.2 | 2.3 | 24.0 | 26.3 | 1.6 | 2.8 | 4.4 |
| Cosumne | 11.7 | 82.0 | 93.7 | 8.0 | 96.0 | 104.0 | 5.0 | 104.7 | 109.7 | 4.6 | 120.0 | 124.6 | 5.9 | 87.0 | 92.9 | 6.9 | 28.0 | 34.9 | 4.0 | 3.0 | 7.0 |
| Ukiah | 2.4 | 54.7 | 57.1 | 2.7 | 58.0 | 60.7 | 1.5 | 62.4 | 63.9 | 2.2 | 76.5 | 78.7 | 3.5 | 73.0 | 76.5 | 3.2 | 36.0 | 39.2 | 3.5 | 10.0 | 13.5 |
| Wheatland | 2.8 | 59.0 | 61.8 | 1.7 | 59.0 | 60.8 | .9 | 64.8 | 65.7 | 1.8 | 80.3 | 82.1 | 2.4 | 84.0 | 86.4 | 3.3 | 55.3 | 58.6 | 2.3 | 9.3 | 11.6 |
| Oregon | 2.1 | 53.0 | 55.1 | 1.5 | 60.0 | 61.5 | 1.2 | 66.0 | 67.2 | 1.8 | 67.0 | 68.8 | 3.4 | 77.6 | 81.0 | 3.5 | 33.3 | 36.8 | 5.4 | 11.0 | 16.4 |
| New York | 10.1 | 66.3 | 76.4 | 8.4 | 71.6 | 80.0 | 6.3 | 85.8 | 92.1 | 5.4 | 100.0 | 105.4 | 6.4 | 78.4 | 84.8 | 8.0 | 30.2 | 38.2 | 9.4 | 9.0 | 18.4 |
| Washington | 1.7 | 50.8 | 52.5 | .5 | 55.5 | 56.0 | .5 | 61.7 | 62.2 | 1.5 | 80.4 | 81.9 | 3.0 | 75.2 | 78.2 | 3.0 | 33.0 | 36.0 | 4.8 | 9.9 | 14.7 |
| Imported (Saaz) | ..... | ..... | ..... | 3.9 | 32.3 | 36.2 | 3.0 | 41.9 | 44.9 | 3.7 | 56.0 | 59.7 | 4.1 | 47.5 | 48.6 | 3.8 | 20.0 | 23.8 | 4.0 | 7.5 | 11.5 |

## COMPARISON OF THE ACID, ESTER, AND SAPONIFICATION NUMBERS OF THE FRACTIONS

The acid numbers of the fractions during each year are most variable; hence difficulty is encountered in attempting a comparison of the oils by this means. From the table it is quite obvious that the oils which in their original conditions possessed free acidity (as indicated by the high acid numbers) show much more decomposition of esters with the liberation

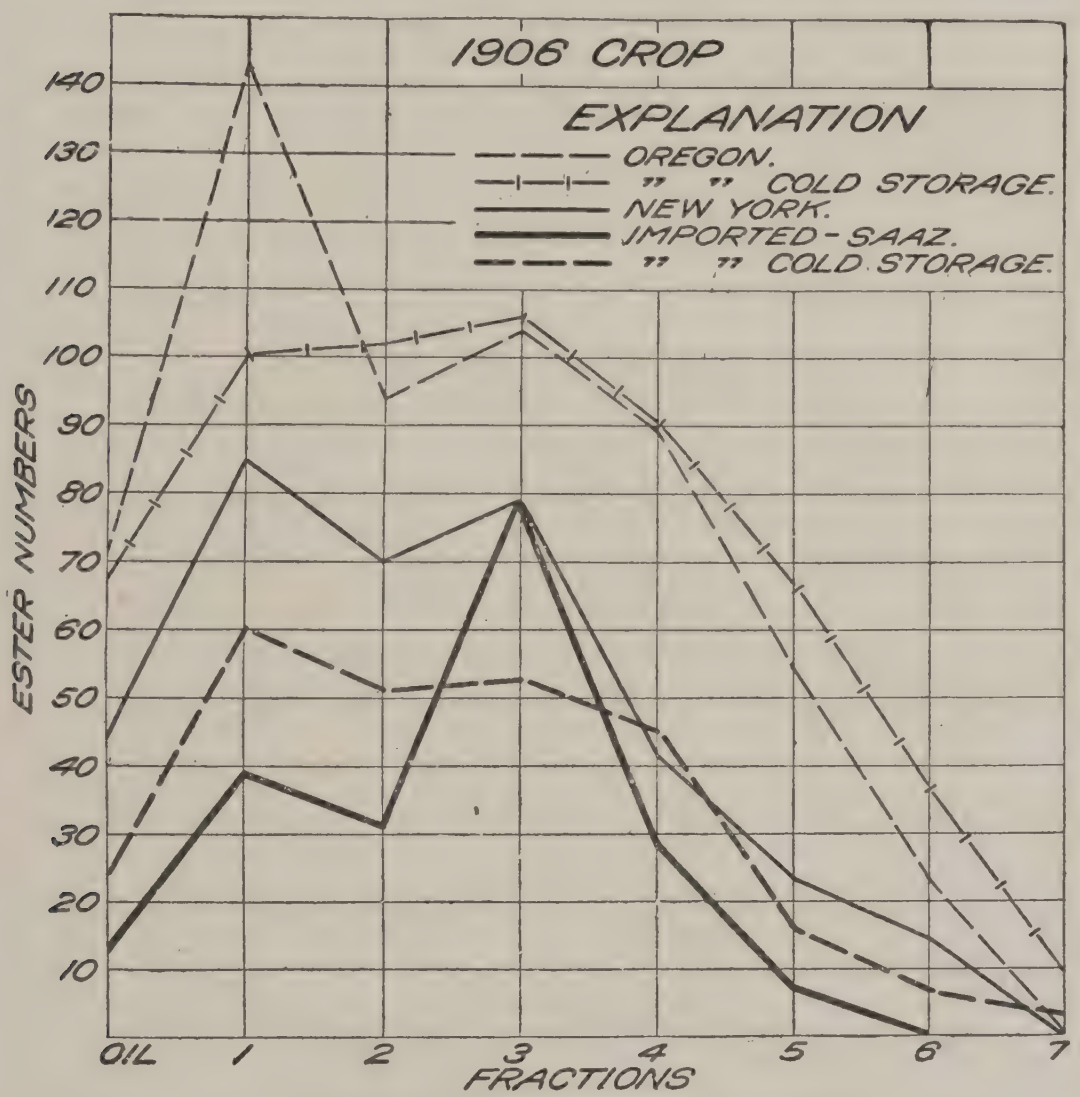



FIG. 10.—Ester-number curves of hop oils from the crops of 1906.

of free acids than do the oils with less acidity or with none. The acid numbers of the fractions of these high-acid oils are in all cases notably higher than those of similar fractions of the other oils. It is therefore very probable that oils with high acid numbers are much less stable than those free from acidity. This may in turn be true of the hops from which the oils were distilled. No particular oil appears to show a regular increase or decrease in acidity of the fractions, hence it is most difficult to draw conclusions from these values.

In direct contrast with the irregularity of the acid numbers of the various fractions there is a remarkable regularity of the ester numbers from year to year. There is also a most notable regularity of increase

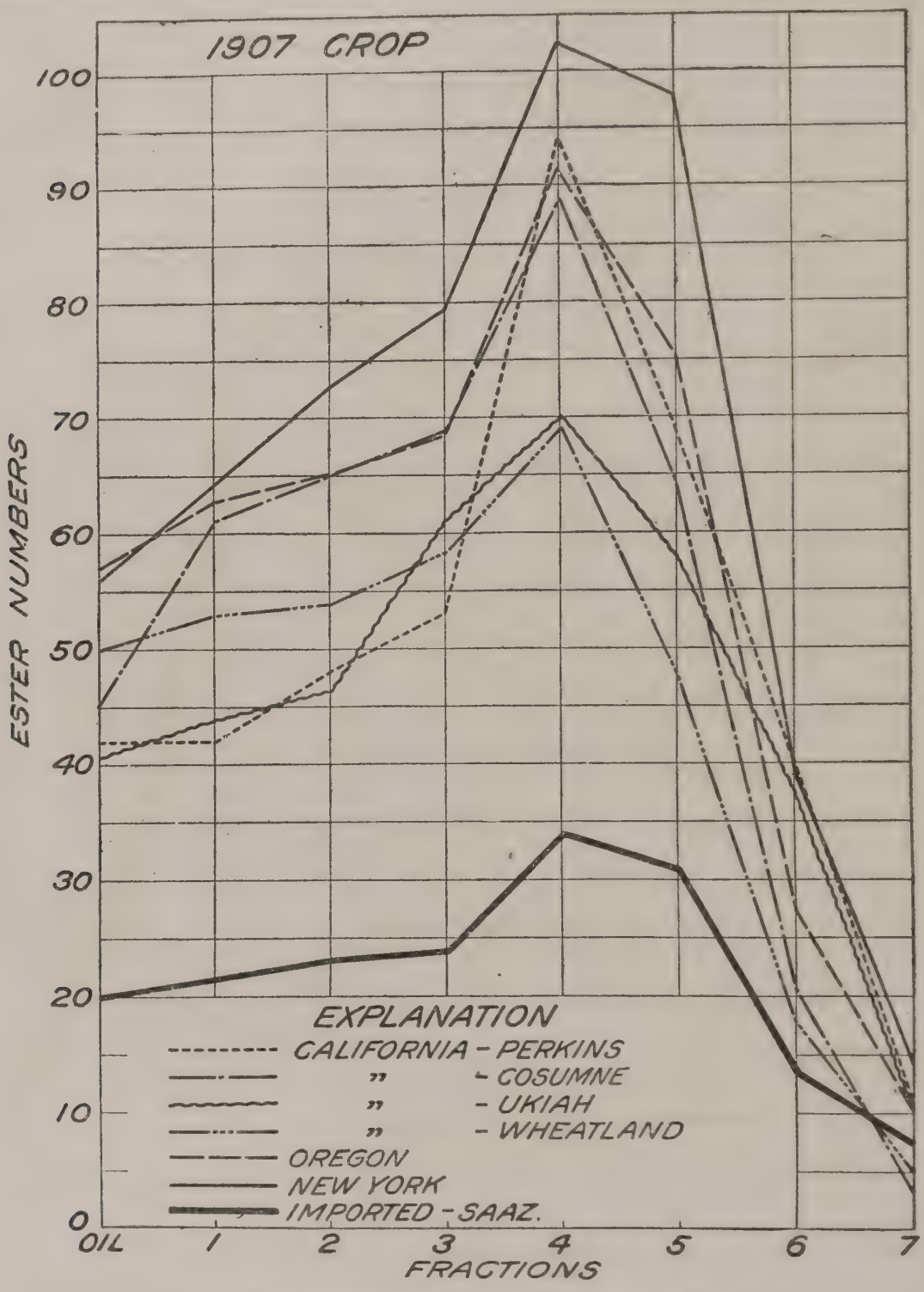



FIG. 11.—Ester-number curves of hop oils from the crops of 1907.

in ester numbers as the particular fractions are approached in which the boiling point most nearly corresponds to those of the esters present. A correspondingly regular decrease is noted in each succeeding fraction from this point to the highest boiling fraction. This is clearly shown by

the curves in figures 10, 11, 12, and 13. These curves show the ester numbers of the original oils and the fractions obtained from each oil, the general direction being the same in each season. Figures 11, 12, and 13 show the great similarity from year to year of the ester numbers and fractions of the oils from the same section. (See also fig. 1.) It will be

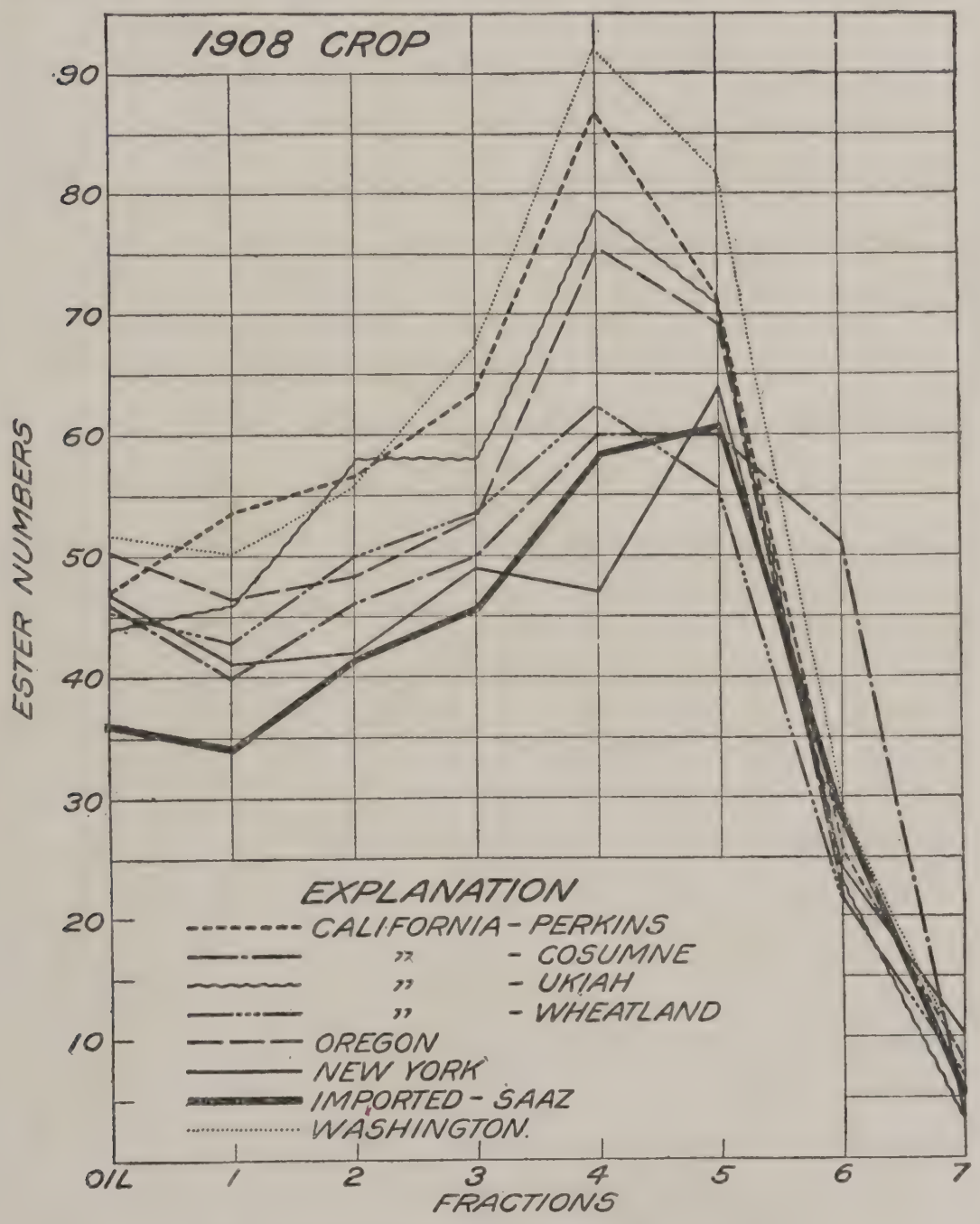



FIG. 12.—Ester-number curves of hop oils from the crops of 1908.

observed that a very close relationship exists between the oils and the fractions from any particular locality. The amount of esters increases as the fractionation proceeds until fraction 4 is reached, after which the quantity decreases until there is practically none in fraction 7. Apparently the oil highest in esters distills between the temperatures of 185° and

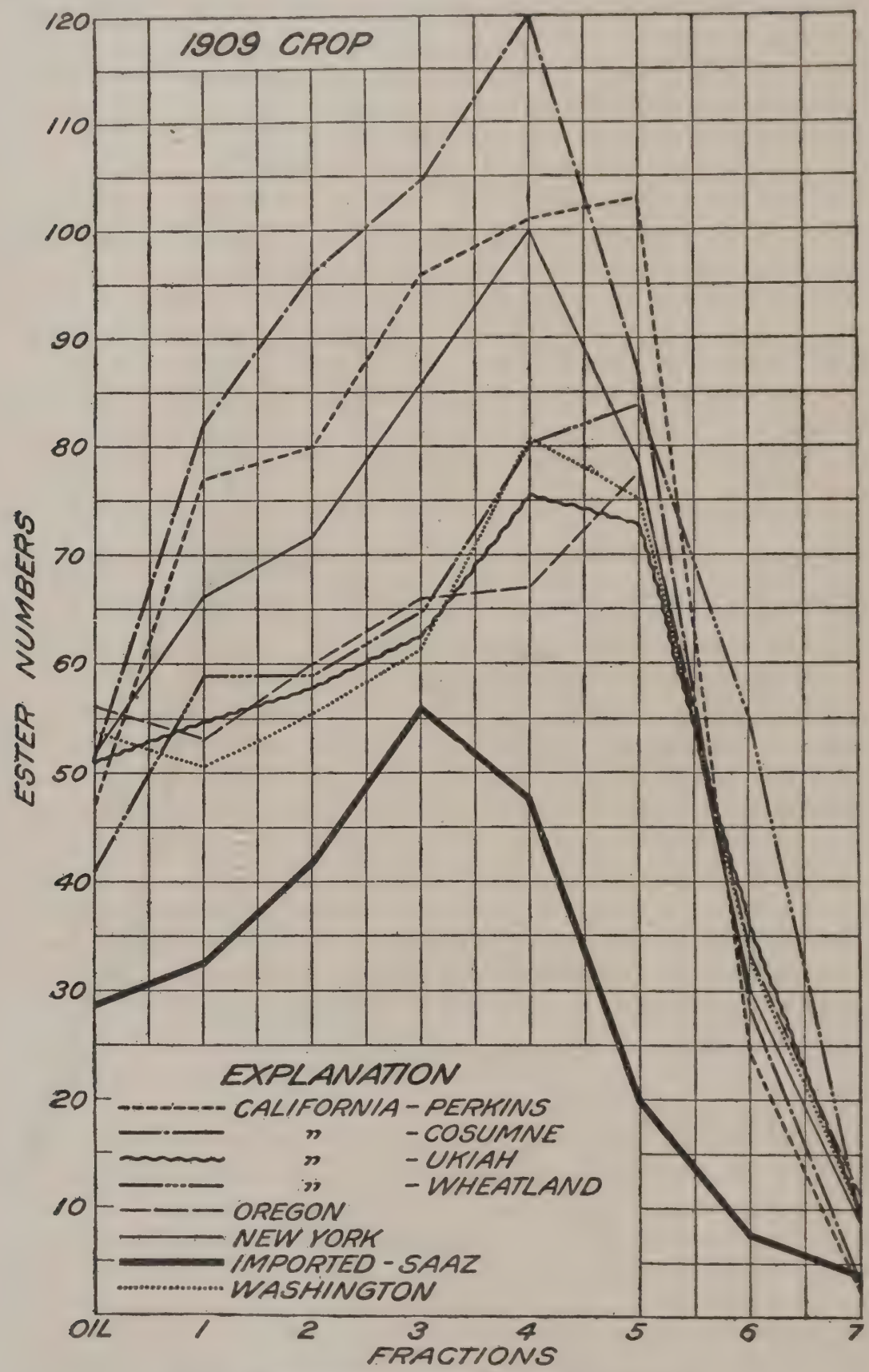



FIG. 13.—Ester-number curves of hop oils from the crops of 1909.

225° C., a considerable amount of esters, however, appearing also in the preceding fractions.

That the fractionation proceeded with fair regularity in each oil is shown by the relationship between the ester numbers of each fraction and the general direction of the curves, which is the same in all the oils.

During the three seasons the curves seem to group themselves according to the source of the hops, those of the oils which differ most in ester content being separate from the others. The course of the curves is therefore, in a sense, an indication of the source of the hops. Throughout the several seasons the curve of the imported hops is conspicuous by its low position as compared with the curves of the oils from the American hops. While it can not be stated with certainty that the oil from the imported hops would continue to show this low ester content, it is very probable that the same differences would continue, since nine samples from five successive seasons all yielded oils poorer in esters than the oils from the American hops during the same seasons.

## CHEMICAL EXAMINATION OF THE OIL

### FREE ACIDS

A small amount of free acids is contained in the oil of hops. In order to identify, if possible, these free acids, the oil was shaken out with an aqueous solution of sodium carbonate. The alkaline liquid was subsequently acidified and distilled with steam. The aqueous distillate, which was acid to litmus paper, was neutralized with sodium hydroxid and precipitated with silver-nitrate solution. By ignition of the dried silver salts, traces of formic and heptoic acids were observed, but the bulk of the volatile acids was identified as valerianic acid, 52 per cent of silver salt being obtained. (Silver valerianate requires 51.6 per cent of silver.) Formic acid was further identified by the formation of a silver mirror from ammoniacal silver-nitrate solution and by the reduction of mercuric chlorid to mercurous chlorid.

### COMBINED ACIDS

After saponifying a quantity of the oil with alcoholic potassium hydroxid, diluting with water, and separating the unsaponified portion of the oil, the resulting alkaline liquid was evaporated to a small volume. Acidification with sulphuric acid caused the separation of an oily layer on the surface of the liquid. The oily acids thus separated had a pronounced odor of some of the higher fatty acids. After separation of the oily layer from the aqueous distillate by means of a separatory funnel, the distillate was shaken out with ether to remove traces of the insoluble fatty acids. The liberation of such a large quantity of oily acids shows that the esters of the oil are for the most part in combination with acids of a higher molecular weight which are oily in character and are sparingly soluble in water.

After neutralization and fractional precipitation with silver-nitrate solution, the aqueous distillate representing the soluble volatile combined acids produced small amounts of silver salts which contained 66 and 71 per cent of silver, respectively. The combined soluble acids therefore consisted largely of formic acid with a trace of acetic acid.

The oily acids previously mentioned, which represented the bulk of the combined acids, corresponded to 5 per cent of the total oil saponified. A small amount of the oily acids, which had a most unpleasant, repulsive odor, was neutralized with potassium-hydroxid solution. The neutralized solution was then precipitated fractionally with silver-nitrate solution. The first two fractions precipitated, which were the smallest and represented the most insoluble salts, yielded 38.5 and 39.2 per cent of silver, corresponding to decylic (caprinic) acid. (Silver salt of decylic acid contains 38.6 per cent of silver.) The third and fourth fractions of silver salts assayed 40.3 and 40.9 per cent of silver. These results correspond very closely to nonoic (pelargonic) acid, whose silver salt contains theoretically 40.7 per cent of silver. Fractions 5 and 6, containing 41.6 and 44 per cent of silver, respectively, had perhaps traces of the preceding nonoic and succeeding lower acids. In all probability a portion of these fractions consisted of octoic (caprylic) acid, which theoretically requires 42.9 per cent of silver. The final fractions both gave 46 per cent of silver, which corresponds well with heptoic (œnanthylic) acid. This acid contains theoretically 45.5 per cent of silver. Based on the total weight of all precipitates, it appears that the oily acids in combination as the esters in the oil of hops consist of about equal proportions of heptoic, octoic, and nonoic acids, with a somewhat smaller content of decylic acid.

Analyzing the fractions of a fractionated oil in another experiment for both free and combined acids, these results were verified in every respect, and additional acids were identified. In the lower boiling fractions butyric acid was identified among the free acids in addition to the formic, valerianic, and heptoic acids previously mentioned.

Heptoic, octoic, and nonoic acids were formed in the free state in the higher boiling fractions, due probably to the splitting off of these acids from the esters at the high heat of distillation. The insoluble acids in all the fractions consisted largely of heptoic and nonoic acids, with some octoic acid, the highest boiling fractions containing two higher acids, decylic and undecylic.

### FRACTIONATION OF SAPONIFIED OIL

The saponified oil remaining after separation of the acids was twice fractionated with a 3-bulb Ladenburg flask, and the results are given in Table VIII.

TABLE VIII.—*Fractionation of saponified oil of hops, showing physical properties of the fractions.*

| Fraction. | Temperature.[a] | Distilled over. | Specific gravity at 22° C. | Rotation in 50-mm. tube. | Refraction ND 22° C. | Remarks. |
|---|---|---|---|---|---|---|
| | °C. | *Per cent.* | | *Minutes.* | | |
| 1..... | Below 160 | 4.0 | 0.8096 | −21.7 | 1.4615 | A large portion of this fraction distilled below 100° C. Strong irritating odor. |
| 2..... | 160 to 165 | 15.5 | .8180 | −21.7 | 1.4710 | Mild aromatic, pleasant yet peculiar odor, unlike any of the common terpenes. |
| 3..... | 165 to 170 | 15.0 | .8093 | −23.9 | 1.4703 | |
| 4..... | 170 to 175 | 2.7 | .8440 | −28.0 | 1.4750 | Pleasant odor, similar to preceding fraction. |
| 5..... | 175 to 185 | 2.2 | .8504 | −33.2 | 1.4752 | More strongly aromatic, pleasant. |
| 6..... | 185 to 195 | 2.1 | .8767 | | 1.4799 | Distilled largely at 195° C. Pleasant hoplike odor. |
| 7..... | 195 to 205 | .9 | .9060 | −45.6 | 1.4836 | Distilled uniformly. Pleasant odor, reminding of hops. |
| 8..... | 205 to 215 | .7 | | | 1.4865 | Distilled mostly from 205° to 210° C. Odor pleasantly aromatic, less fragrant. |
| 9..... | 215 to 245 | 2.2 | .8680 | −45.6 | 1.4818 | Temperature rose rapidly to 245° C. Odor less pleasant. |
| 10.... | 245 to 255 | 2.7 | .8860 | −23.9 | 1.4878 | Distilled uniformly. Odor strong and rather disagreeable. |
| 11.... | 255 to 265 | 5.3 | .8740 | − 2.1 | 1.4871 | Distilled uniformly. Odor strong and unpleasant. |
| 12.... | 265 to 275 | 6.0 | .8850 | +10.3 | 1.4947 | Distilled largely between 265° and 270° C. Not strongly aromatic, disagreeable. |
| 13.... | 275 to 285 | 3.2 | .8870 | + 6.9 | 1.4948 | Disagreeable odor. |
| 14.... | 285 to 295 | 3.8 | .8930 | + 7.6 | 1.4973 | Heavy oil with rather strong, unpleasant odor. |
| 15.... | 295 to 305 | 2.8 | .8940 | + 8.2 | 1.5000 | Heavy oil with repulsive odor. |

[a] Residue 305° and over.

## ALDEHYDE CONSTITUENTS

The initial distillate, which had a most irritating and penetrating action upon the nasal passages, was tested for reducing properties. A silver mirror was readily produced with silver nitrate, and a decolorized solution of fuchsin (magenta solution) became bright red immediately. Lebbin's solution (5 per cent resorcin in a solution of 40 to 50 per cent of sodium hydroxid) produced a deep-red color, characteristic of formaldehyde.

## IDENTITY OF TERPENE HYDROCARBON WITH MYRCENE

The boiling points of the second and third fractions signified a terpenic character, but the odor was unlike that of the usual terpenes. An elementary analysis gave the following results:

*Fraction 2.*—Carbon, 87.5 per cent; hydrogen, 10.7 per cent.
*Fraction 3.*—Carbon, 86.4 per cent; hydrogen, 11.9 per cent.

While it must be remembered that these fractions were not pure, the percentage of carbon and hydrogen nevertheless points to terpene composition, since $C_{10}H_{16}$ requires 88.1 per cent of carbon and 11.7 per cent of hydrogen.

Preparation of the nitrosochlorid and other crystalline terpene derivatives with bromin and halogen acids failed in all cases. This, together with the unusually low specific gravity, pointed to another class of related hydrocarbons belonging to the aliphatic series. Aliphatic hydrocarbons with the same empirical formula, $C_{10}H_{16}$, are termed the "olefinic terpenes." The presence of olefinic terpenes in volatile oils was first observed by Power and Kleber (1895), who isolated a hydrocarbon from oil of bay, which was termed "myrcen." Chapman (1903) mentioned this hydrocarbon as a constituent of oil of hops.

In order to compare the hydrocarbon myrcene of bay oil with the hydrocarbon from hop oil, the properties of the respective compounds were tabulated as given in Table IX.

TABLE IX.—*Comparison of the properties of hydrocarbon from oil of hops with the olefinic hydrocarbon myrcene.*

| Properties. | Hydrocarbon from oil of hops. | Myrcene. |
|---|---|---|
| Boiling point | 165° to 170° C. | 171° C. |
| Specific gravity | 0.8093 at 22° C. | 0.8023 at 15° C. |
| Index of refraction | 1.4703 | 1.4673. |
| Rotation (50-mm. tube) | −23.9′ | Inactive. |
| Color and behavior on standing. | Colorless, becoming slightly yellow and changing to a viscous mass. | Colorless, becoming yellow and resinifying. |

A very close relationship is observed between the boiling point and the specific gravity of the two compounds. The slight discrepancies which exist, especially in the rotation, are probably due to some impurity of the fraction. The most striking characteristic of the hydrocarbon is its instability. The tendency to polymerize was most marked, the respective fractions in the large number of oils fractionated becoming viscous and almost solid in a comparatively short time. This peculiar property was observed by Power and Kleber (1895) in their experiments with the hydrocarbon from bay oil. It was also exhibited in oil of hops, which became viscous, even when the usual precautions were taken against light and air.

Since the properties of the hydrocarbon from the oil of hops show such a close resemblance to those of myrcene, it can be stated with comparative certainty that the terpene hydrocarbon, which represents the large proportion of the lower-boiling constituents, is identical with the olefinic terpene myrcene. By reference to the curves of fractionation of the various oils (figs. 2, 3, 4, and 5) it will be observed that in practically all cases myrcene constitutes the largest portion of the oils.

## IDENTITY OF ALCOHOL AND ESTERS OF HOP OIL WITH MYRCENOL AND ITS ESTERS

If present in the oil, oxygenated constituents should have a tendency to concentrate themselves in the fractions above the temperature of 185° C. Therefore fractions 6, 7, and 8 were analyzed in order to determine their elementary composition. Granting that the separation of constituents is at most only partially effected by fractionation, the determination of the carbon and hydrogen content of these fractions should show the presence or absence of oxygenated compounds. The oxygenated compounds of a saponified oil are usually alcohols of the formula $C_{10}H_{18}O$. The fractions in question gave the following results:

*Fraction 6.*—Carbon, 81.7 per cent; hydrogen, 11.1 per cent.
*Fraction 7.*—Carbon, 80.0 per cent; hydrogen, 10.6 per cent.
*Fraction 8.*—Carbon, 81.4 per cent; hydrogen, 11.3 per cent.

$C_{10}H_{18}O$ requires 77.8 per cent of carbon and 11.7 per cent of hydrogen. The somewhat higher carbon content of the fractions may be explained by the fact that adhering traces of hydrocarbons were not completely separated in the earlier fractions. Oxygenated constituents of the nature of alcohols with the composition $C_{10}H_{18}O$ are strongly indicated in the fractions mentioned.

Since Barbier (1901) states that myrcene is capable of being hydrated chemically with the formation of an alcohol $C_{10}H_{18}O$, called "myrcenol," it was thought that the alcohol of hop oil might be allied to this compound. Furthermore, it is possible that an alcohol like myrcenol could occur in company with the terpene myrcene, from which it is capable of being prepared.

For a further comparison of the above fractions with myrcenol, Table X was prepared.

When it is remembered that the fractions contain admixtures of other constituents incapable of being separated by fractionation, the properties of the fractions compare very favorably with those of myrcenol. Sufficient similarity exists among the various properties, especially the boiling point, specific gravity, and refraction, to indicate the presence of an alcohol similar to myrcenol in the fractions recorded.

In this connection it was deemed advisable to call attention to the esters of the oil of hops, which are present in considerable proportion, and to compare the chief ester fractions of the oil with the acetic ester of myrcenol. As the fractionation of the oil proceeded, it was observed that the esters concentrated themselves in the fractions boiling at 185° to 225° and 225° to 260° C. Although the fractions were by no means pure esters, a comparison of the physical properties with those of the known esters of myrcenol shows that the esters of the oil boil at much higher temperatures than free alcohol. This is readily explained when

cognizance is taken of the fatty acids identified, whose boiling points are exceedingly high. Thus the boiling point of heptoic acid is 221°, of octoic acid 237°, and of nonoic acid 253° C. A combination of these acids with myrcenol, which boils at 213° to 215° C., would tend to produce esters with a boiling point much higher than the alcohol itself. The comparison of the chief ester fractions of hop oil with the acetic-acid ester of myrcenol is given in Table XI.

TABLE X.—*Comparison of the physical and chemical properties of alcohol fractions from hop oil with the alcohol myrcenol.*

| Boiling points of hop oil fractions and myrcenol. | Specific gravity at 22° C. | Rotation in 50-mm. tube. | Refraction at 22° C. | Description. |
|---|---|---|---|---|
| Alcohol fractions from hop oil: | | | | |
| 185° to 195° C. | 0.8767 | | 1.4799 | Nearly colorless; oily liquid with pleasant hoplike odor, becoming viscous on standing. |
| 195° to 205° C. | [b].9060 | [a]−45.6 | 1.4836 | Slightly yellowish liquid with characteristic hop aroma, becoming viscous on standing. |
| 205° to 215° C. | | | 1.4865 | Heavy yellowish oil with agreeable odor of hops, becoming very viscous on standing. |
| Myrcenol, 213° to 215° C. (99° to 101° C. at 10 mm.). | [c].9012 | ..... | [c]1.4778 | Colorless oily liquid, very odorous; polymerizes, slowly becoming viscous. |

[a] Rotation of combined fractions, 185° to 215° C.
[b] Specific gravity of combined fractions, 195° to 215° C.
[c] At 14.5° C.

TABLE XI.—*Comparison of the chief ester fractions of hop oil with the acetic-acid ester of myrcenol.*

| Fractions and ester of myrcenol. | Boiling point. | Description. |
|---|---|---|
| Chief ester fractions from hop oil: | | |
| Fraction 4 | 185° to 225° C. | Slightly yellow oily liquid with agreeable hoplike odor. |
| Fraction 5 | 225° to 260° C. | Pale brownish liquid with strong hop odor. |
| Myrcenyl acetate | [a] 231° C. | Colorless oily liquid with strong odor. |

[a] 112° C. at 10 mm.

It is very probable that esters of the higher acids, as heptoic, octoic, and nonoic, would boil at higher temperatures than myrcenyl acetate. This would necessitate the concentration of the esters in fractions, as shown above. Earlier fractions would also contain some esters, as would later fractions, since the association of lower and higher boiling compounds with the esters would modify their boiling points. In order to show that the esters constitute no small proportion of the oil, curves of fractionation were made of the original oil (containing esters) and the same oil after saponification of the esters (fig. 14).

The curves show directly opposite courses from each other between the temperatures 170° and 260° C. It was between these temperatures that the large proportion of the esters of the original oil distilled over.

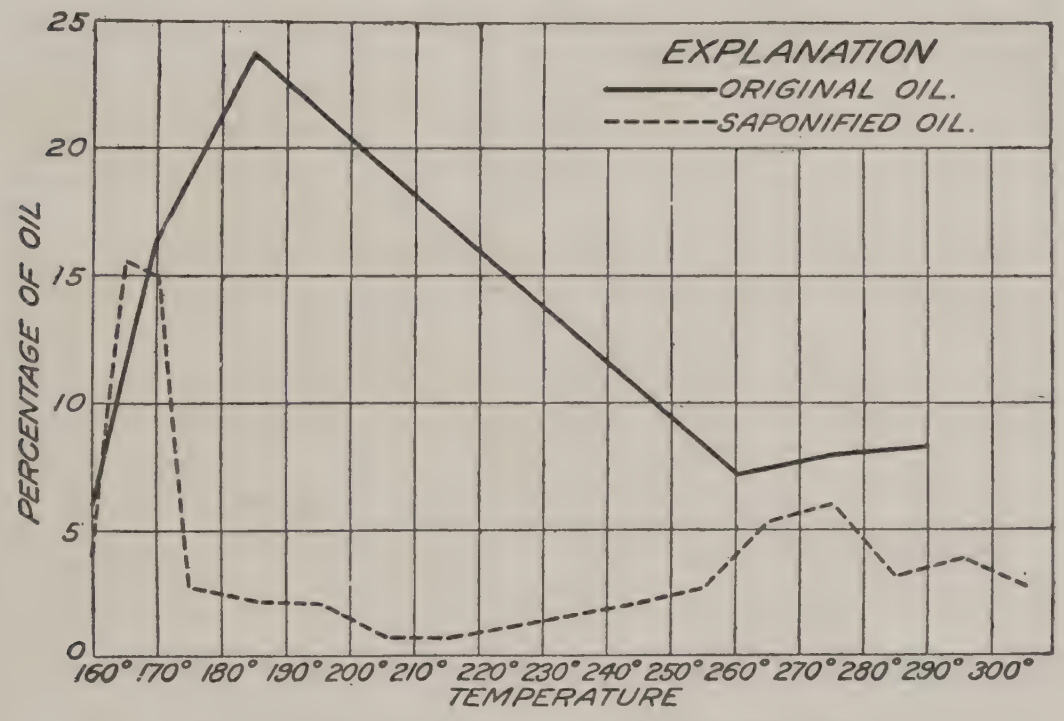



FIG. 14.—Fractionation curves of original and saponified hop oils.

It can therefore be readily perceived that a destruction of these esters by saponification and the consequent removal of the high-boiling acid portion of the esters would tend to flatten the curve of fractionation between these respective points.

The presence of the esters of myrcenol in the oil of hops is not surprising when it is known that the hydrocarbon myrcene, found in the low-boiling fractions, can be readily changed to myrcenol by the action of glacial acetic acid and dilute sulphuric acid. Myrcene bears the same relation to the alcohol myrcenol that camphor does to isoborneol and pinene or that dipentene bears to terpineol. It is very likely that the hydrocarbon myrcene is partly changed to myrcenol by hydration during the formation of the essential oil in the plant, the myrcenol in turn being esterified by the organic acids present.

The reason that it was impossible to quantitatively acetylize the oil was either because there was no free myrcenol present in the oil or because this alcohol would not acetylize without partial decomposition. The latter is probably the case, since in the experiments it was observed that the fractions containing the alcohol and esters were very unstable and readily polymerized to a viscous liquid. Furthermore, these fractions invariably gave negative results when acetylization was attempted.

## IDENTIFICATION OF HUMULENE

Combustion of fractions 9 to 16 gave from 84 to 87.6 per cent of carbon and 11.2 to 11.8 per cent of hydrogen. The theoretical requirement of a sesquiterpene $C_{15}H_{24}$ is 88.1 per cent of carbon and 11.8 per cent of hydrogen. It is probable, therefore, that the fractions consisted of a sesquiterpene with inseparable quantity of alcohols, probably sesquiterpene alcohols which would have a tendency to lower the percentage of carbon. The hydrogen content would remain practically the same for the four classes of compounds—the terpenes, alcohols, sesquiterpenes, and sesquiterpene alcohols. Since the sesquiterpene humulene has been mentioned as a constituent of hop oil, a comparison was made between the physical properties of the high-boiling fractions of the saponified oil and the humulene obtained from different sources, the results being given in Table XII.

TABLE XII.—*Comparison of the physical properties of high-boiling fractions of hop oil with those of humulene.*

| Properties. | Fractions of saponified hop oil. | | | | | Humulene. | |
|---|---|---|---|---|---|---|---|
| Boiling points of fractions and humulene. | 255° to 265° C. | 265° to 275° C. | 275° to 285° C. | 285° to 295° C. | 295° to 305° C. | From hop oil.[a] 261° to 265° C. | From oil of poplar buds.[b] 263° to 269° C. |
| Specific gravity at 22° C. | 0.8740 | 0.8850 | 0.8870 | 0.8930 | 0.8940 | [c]0.8987 | [c] 0.8926 |
| Rotation [d] | −2.1 | +10.3 | +6.9 | +7.6 | +8.2′ | +1° 2′ | [e]+10°48′ |
| Refraction at 22° C. | 1.4871 | 1.4947 | 1.4948 | 1.4973 | 1.5000 | 1.4978 | ..... |

[a] Chapman (1893).
[b] Fichter and Katz (1899).
[c] At 15° C.
[d] Rotation of hop oil fractions determined in 50 mm. tubes.
[e] 200-mm. tube.

Close relationship exists between the physical properties of the fractions of hop oil and those of humulene obtained from both oil of hops and oil of poplar buds. It is possible that traces of sesquiterpene alcohols occur in the fractions of the highest boiling points, since the boiling

points of certain sesquiterpene alcohols correspond very closely to these. Thus, cedrol, the sesquiterpene alcohol obtained from cedar-wood oil, boils at 282°, and santalol, obtained from oil of santal wood, boils at 301° C. The possible presence of sesquiterpene alcohols is also indicated by the low carbon content of these fractions, as shown by the elementary analysis. In order to positively identify the sesquiterpene humulene in oil of hops, three high-boiling fractions (225° to 245°, 245° to 260°, and 260° to 275° C.) of several unsaponified oils were used. Petroleum ether solutions of these fractions were treated with a concentrated solution of sodium nitrite with an equal volume of glacial acetic acid added in small portions and vigorously agitated, the mixture being kept well cooled. In fraction 245° to 260° there appeared blue needle-shaped crystals, which melted at 125° C. This is in accordance with the melting points of humulene nitrosite recorded by Chapman (1895b, p. 783) and by Fichter and Katz (1899), which are, respectively, 120° and 127° C. The characteristic blue coloration of the fraction from which the crystals were obtained was produced in each case.

### APPROXIMATE COMPOSITION OF THE OIL

From the foregoing analysis of the oil of hops it appears that it has approximately the following composition:

FREE ACIDS.—Chiefly valerianic, with traces of formic, butyric, and heptoic acids.

COMBINED ACIDS (*in form of esters*).—Chiefly heptoic (œnanthylic) and nonoic (pelargonic) and somewhat smaller quantities of octoic (caprylic), some decylic (caprinic) and undecylic acids, with traces of formic and acetic acids.

ALDEHYDES.—Formaldehyde in the lowest boiling fraction.

HYDROCARBONS.—Myrcene (olefinic terpene), 30 to 50 per cent. Humulene (sesquiterpene), 15 to 25 per cent.

ESTERS.—Chiefly heptoic, octoic, and nonoic acid esters of the alcohol myrcenol, to the extent of 20 to 40 per cent. From the ester number (44) of the oil in question the amount of esters calculated as the heptoic-acid ester of myrcenol was found to be 21 per cent. If calculated as the octoic or nonoic acid esters, the percentage would be considerably augmented.

ALCOHOLS.—Probably myrcenol and a small proportion of sesquiterpene alcohols.

### RELATION OF THE VOLATILE OIL TO THE SOURCE OF THE HOPS

From the data presented it is clearly evident that the geographical source of hops has a pronounced effect upon the volatile oil and hence also upon the odor of the hops. The oils distilled from hops of different origin have been shown to possess like constituents, which, however, exist in sufficiently varying proportions to impart a decided difference to the properties of the oil. These differences appear to be constant from season to season, not only in the physical properties but also in the more important chemical properties. Most conspicuous among the chemical properties is the ester value, which is closely related to the odor. The ester content is the most influential factor in modifying the odor of the oils and

consequently that of the hops. It is usually acknowledged that hops of foreign origin possess a more agreeable odor than American hops. The difference in odor always seems to be perceptible although the odor is closely related to that of American hops. That the difference in odor is due to a difference in the volatile oil present can scarcely be questioned; in fact, it has been shown that such is the case. This is not, however, the only instance among aromatic plants where geographical source, with the different conditions of climate and soil, shows its effect in the differences in volatile oils distilled from the plants, as, for example, lavender and peppermint. Not only do hops of foreign origin produce oils noticeably dissimilar in some of their properties from the American oils, but hops grown even in separated sections of the United States have a different odor and yield oils with more or less constant differences from year to year.

## CONCLUSIONS

The volatile oil of hops has been shown to consist chiefly of the terpene myrcene, the heptoic, octoic, and nonoic acid esters of the alcohol myrcenol, and the sesquiterpene humulene, with traces of free acids, formaldehyde, and probably some free alcohols. The constituents of chief importance as regards odor are the above-named esters, which constitute a large portion of the oil.

The several oils examined have been found to contain varying proportions of the esters as well as the terpene myrcene and the sesquiterpene humulene. Although no great importance can be attached to the two latter constituents, the variable content of esters is most significant, since the quality of the odor is probably most greatly influenced by them.

Important differences in the oils are apparent not only during any particular season but for several seasons. These constant differences are shown most forcibly in the curves of the physical and chemical properties of the oils. The curves of fractional distillation, which may be said to represent a partial quantitative separation of the chief constituents, bring out strongly the relationship which exists between the hops from any source during one season or several seasons. The optical rotation curves also show this relationship. In general the physical properties of the oils—the fractionation, specific gravity, and optical rotation—show strong similarities which may exist among related oils or strong dissimilarities among unrelated oils. The esters being regarded as the constituents of most importance in affecting the odor of the hops from which the oils were distilled are compared by means of the ester numbers. The curves of the ester content of the various oils and fractions of the oils show at a glance the remarkable differences in the oils from the several geographical sources. The courses followed by the imported oils are most conspicuous because of their constantly lower ester content. More remarkable is the fact that the ester content of the imported oils

remains lower from year to year, practically every sample under observation possessing an exceedingly low ester value. The oils from the California hops are both physically and chemically very similar from season to season. Those from the various sections of California show no important differences in their properties during any particular season, the curves showing considerable parallelism. The Oregon and Washington oils are very similar in their properties, but differ somewhat from the California oils. The New York oils seem to be the most closely related to the foreign oils in all properties, with the exception of the ester content, which is considerably higher. From the standpoint of the increasing ester content the various oils arrange themselves in the following order: Imported, California, Washington, New York, and Oregon, the three latter being very closely related. (See fig. 1.)

No general conclusions can be drawn regarding the possible superiority of any particular oil as compared with any of the other oils. Whether high or low ester content denotes richness or poorness in the quality of the hops, or vice versa, can not be definitely stated. Suffice it to say that from the results obtained it is clear that the geographical source of hops may be indicated by the ester numbers of the oil distilled from the hops, since the experiments show that the ester numbers of the oils from hops of any particular source or season are very similar.

## REVIEW OF PREVIOUS CHEMICAL INVESTIGATIONS OF THE AROMATIC CONSTITUENTS OF HOPS

Reference to the odor of hops was made in literature as early as 1819. Loiseleur Deslongchamps (1819) mentioned the cones of hops as possessing a bitter taste and a strong odor resembling garlic. Hanin (1819) reported that the yellow powder of hops was very tasteful, very odorous, and inflammable, with all the characteristics of a resin. Ives (1821) in an investigation of the properties of hops, referred to the yellow powder of the cones as "lupulin," which, he stated, contained the bitter principle and the aromatic flavor of the hops. Working with samples of 1 dram to 2 ounces of lupulin, no volatile oil was separated, but the aqueous distillate possessed the odor of hops. The following year Payen and Chevalier (1822) found that by distilling the yellow powder (lupulin) a volatile oil was obtained which had the characteristic odor of hops.

The first investigation concerning the nature of the oil was made by Wagner (1853) who distilled the oil directly from the hops instead of from the lupulin. The oil was described as being bright brownish yellow in color with a srtong odor of hops and a burning, slightly bitter taste. The solubility of the oil in water was sufficient to impart a decided hop odor, the solubility being influenced by dextrin, sugar, hop extract, etc. It was stated that the oil contained a terpene, which was likened to camphene, and also an oxygenated constituent, isomeric with borneo camphor.

According to Personne (1854), lupulin when boiled with water produced a volatile oil and a nonvolatile residue. Valerianic acid was identified among the volatile products; also an oxygenated compound called "valerol." Some years later Kuhnemann (1877) distilled hops with steam and obtained an oily substance which he called "hop oil." The oil was described as being a mixture of hydrocarbons and oxygenated compounds, the latter consisting of alcohols and acids. Ossipow (1883)

distilled commercial lupulin and obtained an oil. By experimenting with the oxidation of the oil he identified acetic and isovalerianic acids. Octoic, or caprylic, acid was identified in the aqueous distillate from the oil by means of the silver salt. In the aqueous distillate from the lupulin he also identified butyric and valerianic acids (1886).

It was not until 1893 that Chapman (1893) undertook the study of hop oil and obtained some insight regarding its actual composition. He found in his first experiments that an oil which had been standing for 11 months boiled considerably higher than freshly distilled oil and contained a sesquiterpene as its chief constituent. Continuing his work on the composition of the oil, he concluded after careful fractionation that the sesquiterpene which he had found as the chief constituent was humulene (1894; 1895a). The highest boiling fraction (263° to 266° C.), which consisted chiefly of humulene, represented in most cases about 40 per cent of the oil. The lowest boiling fraction (about 170° C.) also represented a large proportion of the oil and was considered to be a terpene hydrocarbon. The middle fractions had a pleasant odor and consisted of oxygenated compounds. The humulene from the highest boiling fraction of the oil was subsequently investigated by Chapman (1895b) and several crystalline derivatives of this compound were prepared. In a later report (1898) he stated that the oil contained no phenols, aldehydes, or ketones; that it was sparingly soluble in water, but sufficiently so to impart a characteristic odor to the water; also that the oil changed in the air to a viscous mass. In 1903 the investigation was continued (Chapman, 1903), and the lowest boiling fraction (166° to 168° C.) was found to have properties similar to myrcene from oil of bay. One of the higher boiling fractions had a strong odor, which fraction, it was stated, probably contained linalool or esters of linalool. The acids obtained by saponification of the oil were identified by means of their silver salts as valerianic and isononoic acids. In all freshly distilled samples of the oil, myrcene and humulene were said to be present to the extent of 80 to 90 per cent.

Semmler and Mayer (1911) by the preparation of a number of characteristic derivatives established the identity of the terpene from hop oil with the terpene myrcene.

According to Deussen (1911) the humulene of hop oil is very similar in general properties and crystalline derivatives to the sesquiterpene caryophyllene.

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# PRELIMINARY AND MINOR PAPERS

## LESSER BUD-MOTH

By E. W. Scott and J. H. Paine,
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During the spring of 1912, while engaged in apple spraying experiments at Benton Harbor, Mich., the senior author noticed the work of a small larva in the buds of unsprayed apple trees. The injury inflicted was severe in a neglected orchard near the laboratory, and this insect, among others, was the most important factor in the destruction of the entire crop of fruit. Because of the character of the injury, the attack on the swelling buds, and the tying together of the growing leaves, the damage was at once attributed to the eye-spotted bud-moth (*Tmetocera ocellana* Schiff.).

In 1913 a study was made of the life history and habits of this insect, presumably the eye-spotted bud-moth, and experiments with remedial measures were tried. The first discrepancy noticed between the habits of this insect and those of the eye-spotted bud-moth, as recorded in literature, was the fact that the hibernacula were not necessarily situated near the buds, but were to be found in any suitable place upon the limbs. Following this, many other even more striking differences in habits were noted during the course of the season, and the fact was soon impressed upon the writers that they had to deal with an insect whose economic importance had not been recorded in the United States.

The adult moths, upon submission to August C. Busck, of the Bureau of Entomology, were identified as *Recurvaria crataegella* Busck (1903),[1] a species described by him (with no indication of its life history) in 1903 from material submitted by Mr. William Dietz, of Hazleton, Pa., who reared it from hawthorn (*Crataegus tomentosus*). At that time, however, Busck admitted the probability of the identity of his *R. crataegella* and the *R. nanella* of European authors.

Our own observations of the life history of the lesser bud-moth correspond in detail with those of Houghton (1903), who published a short though complete account of the life history of *Recurvaria nanella*. The *R. crataegella* of Busck is therefore to be regarded as a synonym of *R. nanella*, and in support of this decision Busck has recently furnished the following statement:

> *Recurvaria crataegella* Busck (Proc. U. S. Nat. Mus., v. 25, p. 811, 1903) is identical with the European *R. nanella* Hübner, as already suggested in the description. At that time the life history of the species was but fragmentarily known in Europe, and it was deemed the soundest course to give the American form a separate name, even though it was realized that it would probably prove the same as the European species. The subsequent careful study of the life history in Europe by J. T. Houghton and in this country removed all doubt about the synonymy.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 162.

Stephens (1834) recorded *Recurvaria nanella* as "not very uncommon in gardens within the metropolitan district, frequenting the trunks of apple trees in June and the beginning of July."

Stainton (1854) recorded the larva as feeding in May on the pear, making a gallery across the flowers with pieces of the petals and stamens interwoven with silk.

Rössler (1871–72) observed the tying together of the young leaves of fruit trees by larvæ of *Recurvaria nanella* and its effect in hindering the development of the new leaves. The insect was present in such large numbers as to attract the attention of the public to the deformed trees and to arouse the fear that serious harm would result. In view of the fact that the larva was so small, ate so little, and did not attack the blossoms, Rössler considered that it was not to be feared.

Houghton (1903) published an account of the life history and habits of *Recurvaria nanella* from an economic point of view, as observed by him in England. His attention was directed to the insect in an apricot orchard, where the crop had been practically destroyed by it in previous years. He was the first to note the fact that the larva, after hatching, passes the time before hibernation as a miner in the leaf. He also observed that it was the habit of the larvæ to bore into the swelling buds in the spring. The larvæ appeared in swarms on peaches and apricots and less commonly on cherries and plums. He mentions the different colors assumed by the caterpillars as they near maturity, and this observation corresponds with our own.

The distribution of *Recurvaria nanella* in Europe is given by Staudinger and Rebel (1901) as follows: Central Europe, Sweden, northern Spain, southern France, central and northern Italy, Dalmatia, and southwestern Russia.

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# [illegible]

By James R. [illegible]

[illegible]
Bureau of Plant Industry

The opportunities afforded by the region for continued fungous pursuit in the investigations in forest pathology in the great forest areas of the Northwest, have enabled the writer to collect the fungi of these regions with considerable completeness. In studying up the specimens, collected, and have acknowledge of a large number of species of [illegible] fungi collected during the season of 1907 throughout Washington, Idaho, Oregon, and British Columbia, some new and highly interesting fungi, not heretofore reported, have come to light. Brief descriptions of the distinguishing features of two of these species follow:

*Fomes pinicola* n. sp.—Sporophores hard, woody, very irregular; pileus [illegible] sessile or effused, sometimes broadly spreading or typically resupinate. The resupinate sporophores are often a foot or more in length. Pileus [illegible] to 15 cm. by 6 cm. by 4 cm. The surface is usually [illegible] a velvety or tomentose zone bearing distinctly engraved, yet always more or less wavy, sharply crowded and furrowed in old age, in other [illegible] becoming finely [illegible] pubescent, whitish, sometimes [illegible], with narrow dark-brown [illegible] zones, later becoming blackened, roughened, and cracked by the numerous annual growths, which are wavy in width. Pileus [illegible] tough, corky, [illegible] finished, 2 to 5 mm. long each annual [illegible] much longer in resupinate forms, usually [illegible] 1 to 2 mm. long each annual [illegible] much longer in resupinate forms, usually [illegible] millimeters wide, varying in size 2 to 3 to 5 millimeters, edges [illegible] dense, [illegible] somewhat [illegible], [illegible] annual, [illegible] 1 to 2 [illegible] [illegible] [illegible]

clearly shown in Plate IX, figure 5, where the successive stages of the rot in this host are illustrated. The illustrations show the cellulose pockets in their natural size. As in case of other wood-destroying fungi, the rot varies to a limited extent, according to the anatomy and chemical contents of the wood of the host.

The rot produced by *Fomes conchatus* is entirely different. Conspicuous cellulose pockets are never formed, but the decay is frequently characterized by the appearance in close union of two distinct phases of the decomposition, depending, as it seems, on the physical and chemical nature of certain parts of the wood structure. A yellowish white rot first appears which may remain more or less permanently alongside other areas, continuing in the decomposition, later breaking up in irregular areas when dried.

The hard, black encrusting surface sometimes assumed by *Fomes conchatus* is never present in *F. putearius*. Neither does the context become as hard and woody. The spines, although present in considerable number in *F. putearius*, are not a conspicuous character. Those of *Fomes conchatus* are much more abundant and larger.

Type locality.—Priest River, Idaho; Kaniksu National Forest.

Habitat.—Dead coniferous wood.

Range.—Throughout the Northwest, most abundant in the white-pine zone.

Specimens have been examined from practically all the main forest regions.

**Trametes setosus,** n. sp.—Sporophores pileate or entirely resupinate, depending upon its position on the substratum. The resupinate forms have sharply defined sterile margins and are usually found on the underside of logs, where they may extend for a distance of a foot or more. The distinctly sessile pileate forms are usually free from each other, but may be connected by the resupinate portion, occasionally narrowed at the point of attachment, mostly thickened at the base, rarely applanate or conchate, averaging 1 by 2 by 2 cm. Surface minutely tomentose, becoming smooth or weathered in old specimens, zonate, rich dark brown, uneven; margin thick, of lighter color, entire, becoming slightly serrate in old age, slightly sterile; context ferruginous or fulvous, spongy to corky, slightly zonate, particularly in old specimens; tubes long, often filled with a grayish mycelium, 1 to 1.5 mm.; mouths small, mostly angular, occasionally labyrinth-like, 3 to 6 to a millimeters, edges thick, tomentose; spores hyaline, 4 to 5 by 3 $\mu$. The character that distinguishes the species from all of its near relatives is the immense number of long dense brown setæ lining the interior of the tubes. In no other species known to the writer is this character so distinctly pronounced. The longest spines measure 41.45 $\mu$, the shortest about 22.16 $\mu$, with an average of 30.46 $\mu$. The nature and immense number of these setæ may be determined by a study of Plate X, figure 11.

The fungus shown in Plate X, figures 6 to 11, was first collected in the Kaniksu National Forest near Priest River, Idaho, on fallen *Pinus monticola*. This tree is the principal host, although the fungus occurs occasionally on the wood of other trees but always on conifers. *Polyporus gilvus* Fr., which seems to be the nearest relative and is usually found on the wood of deciduous trees, has not, so far as the writer is aware, been collected in the West.

*Trametes setosus*, on account of its abundance, causes serious damage to fallen merchantable timber in forest-fire areas. The chemical action of the mycelium on the wood is to reduce the lignin principally in the spring wood, leaving a cellulose ring alternating with sound autumn wood (Pl. X, fig. 10), which causes the annual rings to separate.

Type locality.—Priest River, Idaho; Kaniksu National Forest.

Habitat.—Dead coniferous wood.

Range.—Throughout the white-pine (*Pinus monticola*) belt of the Northwest. Specimens were collected in all the principal forest areas from Vancouver, B. C., to Montana.

Type specimens of both species have been deposited in the Office of Investigations in Forest Pathology and in the Pathological Collections of the Bureau of Plant Industry, Washington, D. C.

## PLATE IX

Fig. 1.—*Fomes putearius* from the wood of *Larix occidentalis*, showing a typical pileate fruiting structure; also a section through the resupinate form.

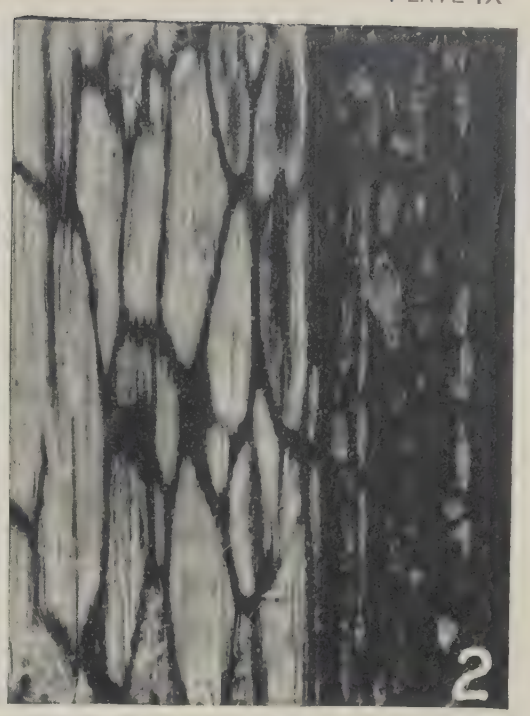
Fig. 2.—*Larix occidentalis*, showing decay of the wood by *Fomes putearius*.

Fig. 3.—*Pinus ponderosa*, showing disintegration of the wood by *Fomes putearius*.

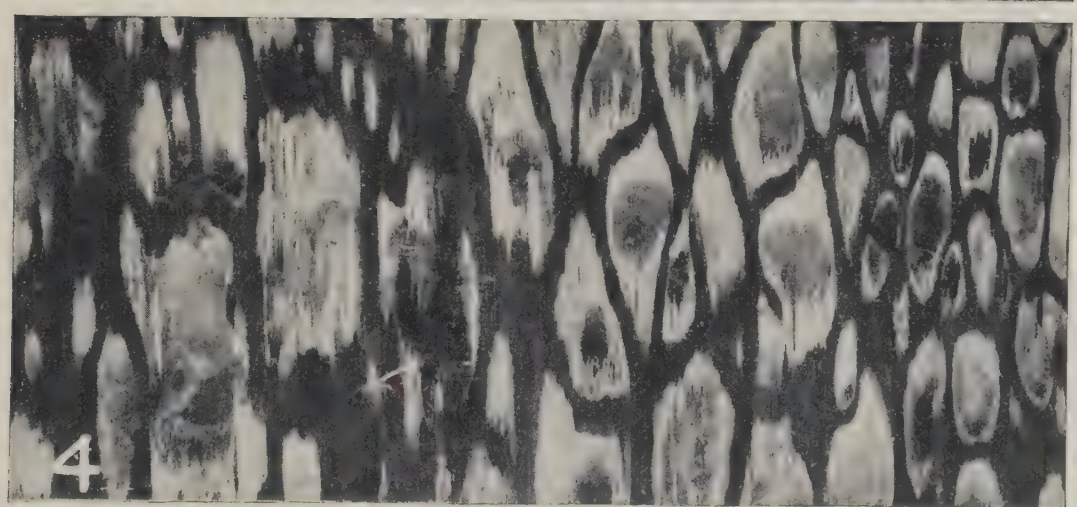
Fig. 4.—*Pseudotsuga taxifolia*, showing the typical decay caused by *Fomes putearius*.

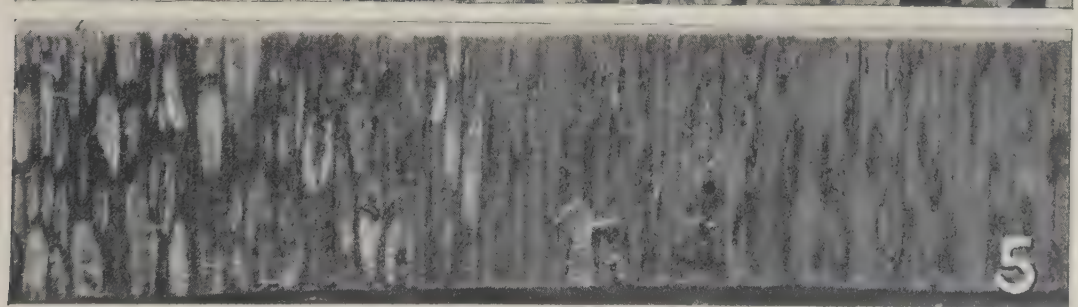
Fig. 5.—*Picea engelmanni*, showing the different stages of the decay caused by *Fomes putearius*.

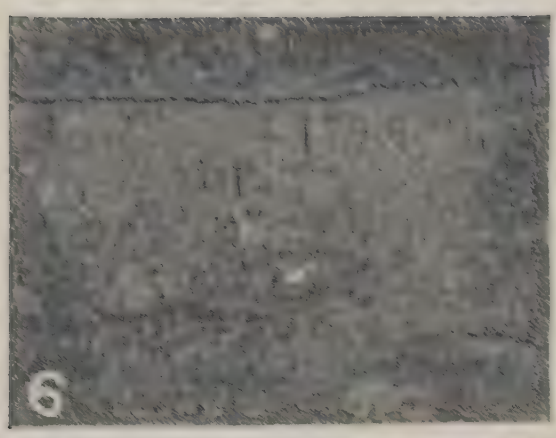

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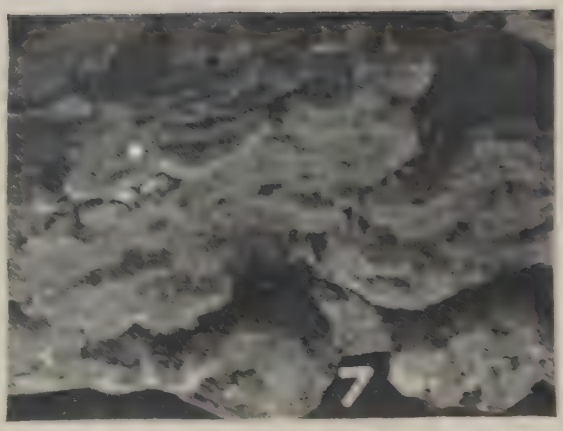

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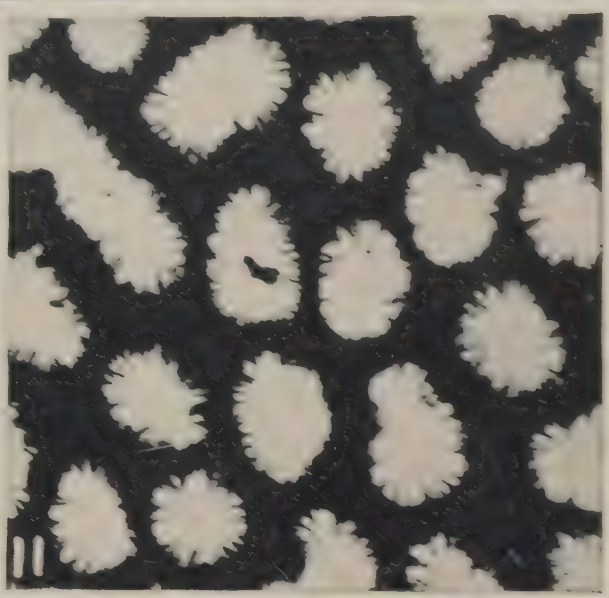

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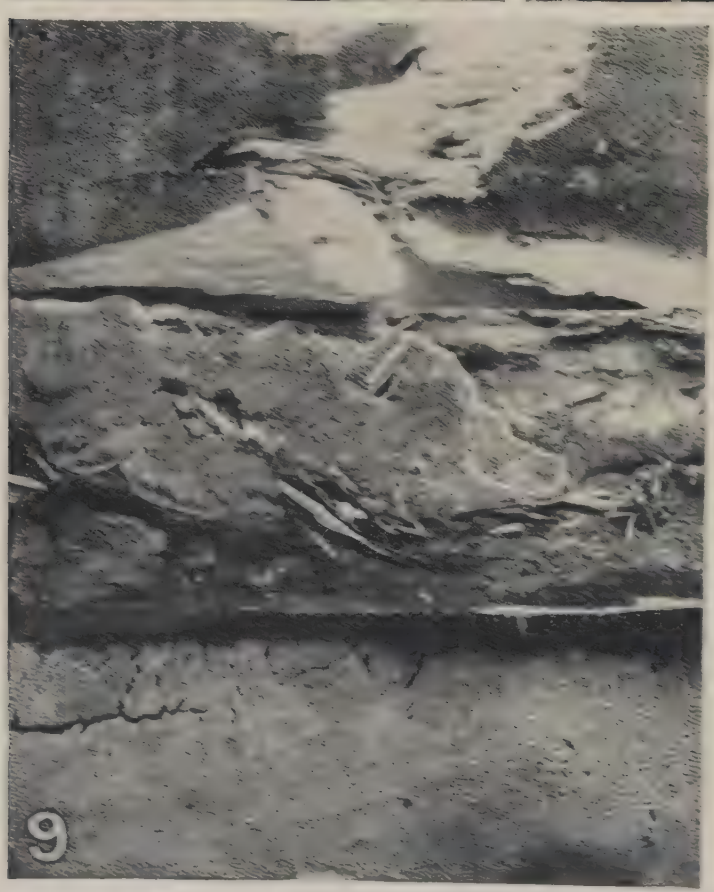

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PLATE 2

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# BACTERIA CONCERNED IN THE PRODUCTION OF THE CHARACTERISTIC FLAVOR IN CHEESE OF THE CHEDDAR TYPE[1]

*By* ALICE C. EVANS, *Bacteriologist, Dairy Division, Bureau of Animal Industry,* E. G. HASTINGS, *Bacteriologist, Wisconsin Agricultural Experiment Station, and* R. B. HART, *Chemist, Wisconsin Agricultural Experiment Station*

In a previous publication (Hastings, Evans, and Hart, 1912)[2] a summary of the knowledge concerning the bacteriology of Cheddar cheese was presented together with the results that were obtained by the authors in an effort to extend the knowledge concerning the flora of this type of cheese. It was shown, as had previously been demonstrated by numerous investigators, that *Bacterium lactis acidi* is an important factor in the making and ripening of Cheddar cheese. The lactic acid formed aids in the curdling of the milk by the rennet, and the acid formed in the curd assists in the expulsion of whey therefrom. The combination of the acid with the paracasein so changes the nature of the curd that the pieces readily coalesce or "mat." The lactic acid also activates the pepsin of the rennet extract, enabling it to act on the paracasein, with the formation of soluble products. The acid reaction of the cheese is maintained during the ripening period and thereafter, thus inhibiting the development of putrefactive bacteria which otherwise would soon destroy the commercial value of the cheese.

It was further shown that lactic bacilli[3] occur in all Cheddar cheese in numbers approximating those of the ordinary lactic bacteria, *B. lactis acidi*, and that coccus forms are also of constant occurrence.

At the beginning of the ripening period the ratio between *Bacterium lactis acidi* and lactic bacilli can, in a general way, be expressed as 99 to 1. This ratio gradually changes as the ripening progresses until in cheese 3 to 6 months old the ratio is reversed. It is apparent that the larger part of the growth of the *B. casei* group must take place after the fermentation

[1] Work of Department of Agriculture in cooperation with Wisconsin Agricultural Experiment Station.

[2] Bibliographic citations in parentheses refer to "Literature cited," pp. 191–192.

[3] This group of lactic-acid-forming organisms appears in the literature under various names, the most common being "lactic bacilli," "*Bacterium bulgaricum*" or "*Bacillus bulgaricus*," and "*Bacterium casei*." The name "*Bacterium casei*" will be used in this article.

of the sugar in the cheese by *B. lactis acidi*. Their continued persistence in large numbers must be due to continued growth for a considerable period or else to their greater resistance to the environment. But little definite information was presented in the bulletin mentioned concerning the occurrence of coccus forms, because of the confusion then existing in the differentiation of some varieties of this group from the *B. lactis acidi* group.

It was recognized that we were not dealing with specific organisms but rather with great groups. In making cheese of the Cheddar type from pasteurized milk it was found that the typical flavor did not develop. The milk, after pasteurization, always contained organisms of the *Bacterium lactis acidi* group, of the *B. casei* group, and coccus forms, and there was added to the milk a starter containing an organism of the *B. lactis acidi* group. In order to obtain a more complete knowledge concerning the biological factors present in Cheddar cheese and their rôle in the development of flavor and possibly to determine the cause of lack of flavor in cheese from pasteurized milk, a more detailed study has been made of cheese both from raw and from heated milk, with the results as presented in this paper.

In continuation of the above-mentioned work, bacteriological analyses of many more cheeses have been made in order to determine more fully the distribution of the groups in ripening cheese; and a detailed study of the pure cultures obtained has been carried out along new lines with the view of correlating the presence of certain types with desirable or undesirable flavor production.

## METHODS OF EXAMINATION

In the previous work the pure cultures were obtained by isolation from lactose-agar plate cultures and from dilution cultures made in small flasks of sterile milk. These milk cultures were inoculated from a dilution of a cheese emulsion, the dilution increasing from flask to flask by a ratio of 10. The above methods have been modified in the present study. The dilution cultures were made in milk to which was added 1 gram of peptone, 1 gram of dextrose, and 200 c. c. of water per liter. This was found to be more favorable than plain milk for the development of some of the cheese organisms. An effort was made to obtain two pure cultures for study from each series of dilution cultures; viz, the predominating organism of the combined *Bacterium lactis acidi* and coccus groups and the predominating organism of the *B. casei* group. The former was obtained by plating the culture from the highest dilution of the cheese which shows a growth after two days' incubation; the latter was obtained after three weeks' incubation. The flask cultures were then titrated to determine the highest dilution which contained *B. casei*. All milk cultures which titrated more than 1.15 per cent of acidity were further examined for the *B. casei* group. The long incubation usually killed

the other groups of cheese organisms, so that a subinoculation into tubes of litmus milk containing dextrose and peptone resulted in the growth of the lactic bacilli alone. The culture was then purified by plating.

The plate cultures of the cheese dilutions were made in casein agar (Ayers, 1911), to which 1 per cent of dextrose was added; they were incubated at 37° C. for two days and then kept at room temperature for four days. In order to obtain the percentage of the various cheese organisms which develop upon the casein-dextrose-agar plates, a portion containing 10 colonies was circumscribed, and the inclosed colonies were fished off into litmus milk. When growth appeared in the milk cultures a microscopic examination was made. Those cultures from a single plate which caused the same changes in litmus milk and were of the same morphology were considered of the same variety. Representatives of every variety were studied in detail and referred to their respective places, according to the classification given below.

This method of studying only 10 colonies from the plate cultures at one analysis and then reducing this number for more detailed study to a single representative of those cultures which are similar in their morphology and their action on litmus milk was intended to give a broad view of the flora of cheese rather than a detailed one. A more intensive study of the cheeses selected for analysis would necessarily reduce the number which could be examined. Inasmuch as the flora of normal Cheddar cheese will differ greatly in a detailed study, the methods used, which allow the examination of a large number of cheeses and give a rough picture of the cheese flora, were considered the best adapted to increase our knowledge in its present stage.

## FERMENTATION TESTS FOR THE CLASSIFICATION OF CHEESE ORGANISMS

The cultural characteristics, morphology, and the few biochemical reactions which are ordinarily considered in classification of bacteria were found to be inadequate traits for distinguishing one variety of cheese organism from another of the same group. Therefore the system of classification based upon the fermentation of various test substances which has been used by Gordon (1905) and other English investigators and by Winslow (1912) and his colleagues for the classification of the Coccaceæ and which was also used by Rogers and Davis (1912) for classifying the lactic-acid bacteria of the *Bacterium lactis acidi* type was adopted, with modifications suited to the problem in hand, for the classification of cheese organisms.

A sugar-free broth was made of 10 grams of compressed yeast, 10 grams of peptone, and 5 grams of dibasic potassium acid phosphate per liter of water. To this mixture was added 1 per cent of the test substance. At first inoculations were made into dextrose, lactose, galactose, sucrose, salicin, mannit, glycerin, inulin, starch, and raffinose broths with all the

groups of cheese organisms. After many cultures had been submitted to these tests it was found that some of the substances had no differential value. All of the cultures produced acidity from some of the substances, while none of the cultures, or exceedingly few, produced acidity from other substances. Moreover, not all of the test substances which proved of value in differentiating into varieties the members of the coccus and *Bacterium lactis acidi* groups were equally valuable in the study of the *B. casei* group.

After a preliminary study of 250 cultures of the cheese organisms on the 10 test substances with quantitative determinations of the amount of acid produced in the dextrose broth, some of the substances were discarded. The study was continued on 5 of the test substances differing for the various groups of organisms.

It now includes approximately 1,000 cultures of cheese organisms isolated from 37 different raw-milk cheeses, which represent every stage of the ripening period. The test for acid was made after 10 days' incubation at 37° C. Small squares of alkaline litmus paper were placed in the cultures. If the color in several cultures of positive and negative reactions is compared this method will detect very slight increases in acidity.

This method of testing the production of acid with litmus paper differs from that of other investigators who have made use of the fermentation tests for the classification of bacteria. These investigators have determined by titration the amount of acid produced in the various broths and have considered these data also in their classification. But in any work of the nature of the problem under discussion it is important that the first stages should be a comprehensive survey of all of the factors which may influence the final result rather than a more intensive study of any one or more factors which attract attention at first sight. The methods which have been followed in the classification of cheese organisms were chosen with the purpose of excluding as much as possible of the routine work, which appeared to be of minor importance for this study in its present stage, in order to extend it over a larger number of samples of cheese. For this reason the fermentable substances employed for the tests were reduced to the smallest number which appeared adequate for the differentiation. And the titration of the acid produced, which in the preliminary study was made in the dextrose broth alone, was entirely omitted in the major part of the study. Rogers and Davis (1912) have adopted an increase of 1 per cent of normal acid as the division between fermentation and nonfermentation. In the case of lactose, salicin, sucrose, mannit, and inulin the reddening of alkaline litmus paper gives practically the same results as far as the division into fermenters and nonfermenters is concerned, for in broths containing these substances the reaction is almost always negative or decidedly positive, with the development of a considerable acidity. If there is a development of acidity in glycerin, the reaction is often slight. The titration of a num-

ber of such cultures showed that the litmus paper will detect increases of acidity as low as 0.5 per cent; these were recorded as positive. Hence, in the comparatively few instances in which the development of acid is slight the results may sometimes be recorded as positive in this study, where other investigators would have recorded them as negative.

The amount of acid produced is regarded as unimportant in the classification of the cheese organisms. The consideration of degrees of acidity would greatly complicate a system which, if it is to be valuable in the study of the cheese problem, should rather be made as simple as possible.

## CLASSIFICATION OF THE BACTERIUM LACTIS ACIDI AND COCCUS GROUPS

In the present study an especial effort has been made to differentiate the *Bacterium lactis acidi* and coccus groups. Since they were submitted to the same tests, the classification of these groups will be considered together. The organic substances which were adopted as the most useful test substances for the coccus and *B. lactis acidi* groups are lactose, salicin, mannit, sucrose, and glycerin. The morphology, the growth on plain agar slopes, and the growth in litmus milk were also considered.

Cultures in which some or all of the cells are elongated and in pairs and which curdle litmus milk with the reduction of the litmus characteristic for *Bacterium lactis acidi* were classed in that group. The Streptococcus group includes all cultures in which the cells are spherical and arranged in pairs or in chains. The cultures of this group do not give the reduction of litmus which is characteristic for the *B. lactis acidi* group. Most of the cultures of the Micrococcus group produce a heavy growth upon the agar slope, which is often of some shade of yellow. They are the members of the Coccaceæ which divide in two planes; consequently the cells appear in pairs, fours, or bunches.

In Table I the sign ± indicates that individual cultures of the given variety may produce acid from the substance in question; or they may fail to produce acid. The test substances are arranged in the table according to their order of availability. For example, lactose is fermented by the greatest number of cultures, and mannit is fermented by the least number of cultures of both groups of the Coccaceæ.

TABLE I.—*Classification of the groups of cheese organisms*

BACTERIUM LACTIS ACIDI

| Variety No. | Production of acidity in broth containing— | | | | |
|---|---|---|---|---|---|
| | Lactose. | Salicin. | Mannit. | Sucrose. | Glycerin. |
| a | + | − | − | − | − |
| b | + | + | − | − | − |
| c | + | + | − | + | − |
| d | + | ± | + | ± | − |

TABLE I.—*Classification of the groups of cheese organisms*—Continued

STREPTOCOCCUS

| Variety No. | Production of acidity in broth containing— | | | | |
|---|---|---|---|---|---|
| | Lactose. | Salicin. | Sucrose. | Glycerin. | Mannit. |
| a | − | − | − | − | − |
| b | + | − | ± | ± | − |
| c | + | + | ± | ± | − |
| d | + | ± | ± | ± | + |

MICROCOCCUS

| Variety No. | Production of acidity in broth containing— | | | | |
|---|---|---|---|---|---|
| | Lactose. | Sucrose. | Glycerin. | Salicin. | Mannit. |
| a | + | ± | ± | − | − |
| b | + | ± | ± | + | − |
| c | + | + | − | − | + |

All organisms which have been found constantly in cheese are included in the four groups *Bacterium lactis acidi*, Streptococcus, and Micrococcus groups, together with the *B. casei* group, which will be discussed later. The different varieties of all four groups produce acid in milk. Spore-forming organisms occur in very small numbers or are entirely absent. The same thing is true of liquefying organisms. Whenever they are present in sufficient numbers to have an appreciable influence in the ripening, it is to the detriment of good flavor.

In the classification of the *Bacterium lactis acidi* group, variety "a" corresponds to group "A" in Rogers and Davis's classification of the lactic-acid bacteria (1912). Varieties "b," "c," and "d" are all included in their group "B," which also includes other varieties which were not found among the cheese organisms and are therefore not mentioned in this work.

## CONSTANCY OF REACTION OF THE CULTURES OF THE BACTERIUM LACTIS ACIDI AND COCCUS GROUPS TO THE TEST SUBSTANCES

It is obvious that the accuracy of this method, in so far as it is a guide for the classification of an individual culture, depends on the constancy of the reactions to the tests. It is the opinion of those who have made a comprehensive study of this method that the fermentation reactions on the test substances are on the whole remarkably constant for any given strain, and therefore they are of value in classification. Representative cultures of the varieties of the coccus and the *Bacterium lactis acidi*

groups have been maintained on agar slopes, with transfers made every month or six weeks. The constancy of the reactions of these cultures on the five organic substances has been tested, and the results on salicin, mannit, sucrose, and glycerin are given in Table II. The results on lactose are omitted, because they are always positive.

TABLE II.—*The constancy of the Bacterium lactis acidi and coccus groups in their action upon test substances*

BACTERIUM LACTIS ACIDI

| No. of culture. | Production of acid in broth containing— | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salicin. | | | | | Mannit. | | | Sucrose. | | | Glycerin. | | |
| | First test. | Age of culture at time of second test. | Second test. | Age of culture at time of third test. | Third test. | First test. | Second test. | Third test. | First test. | Second test. | Third test. | First test. | Second test. | Third test. |
| | | *Months.* | | *Months.* | | | | | | | | | | |
| 1 | − | 8 | − | 12 | − | − | − | − | − | − | − | − | − | − |
| 2 | − | 3 | + | ..... | ..... | − | + | ..... | − | − | ..... | − | − | ..... |
| 3 | − | 9 | + | 12 | + | − | + | + | − | − | − | − | − | ..... |
| 4 | + | 1 | + | ..... | ..... | − | − | ..... | − | − | ..... | − | − | ..... |
| 5 | + | 4 | + | 8 | + | − | − | + | − | − | − | − | − | − |
| 6 | − | 4 | + | 8 | + | + | + | + | − | − | − | − | − | − |
| 7 | + | 4 | + | 8 | + | + | + | + | − | − | − | − | − | − |
| 8 | + | 2 | + | ..... | ..... | + | + | ..... | + | + | ..... | − | − | ..... |

STREPTOCOCCUS

| No. of culture. | Production of acid in broth containing— | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salicin. | | | | | Sucrose. | | | Glycerin. | | | Mannit. | | |
| | First test. | Age of culture at time of second test. | Second test. | Age of culture at time of third test. | Third test. | First test. | Second test. | Third test. | First test. | Second test. | Third test. | First test. | Second test. | Third test. |
| | | *Months.* | | *Months.* | | | | | | | | | | |
| 9 | − | 10 | − | 14 | − | − | + | + | − | − | − | − | − | − |
| 10 | − | 10 | − | 14 | − | − | − | + | − | − | + | − | − | − |
| 11 | − | 6 | − | ..... | ..... | + | + | ..... | − | − | − | − | − | − |
| 12 | − | 5 | − | ..... | ..... | + | + | ..... | + | + | ..... | − | − | ..... |
| 13 | − | 9 | − | ..... | ..... | + | + | ..... | + | + | ..... | − | − | ..... |
| 14 | + | 9 | + | 18 | + | − | − | − | − | − | − | − | − | − |
| 15 | + | 4 | + | 8 | + | − | − | − | + | + | + | − | − | − |
| 16 | + | 4 | + | 8 | + | + | + | + | − | − | − | − | − | − |
| 17 | + | 5 | + | ..... | ..... | + | + | ..... | − | − | ..... | + | + | ..... |
| 18 | − | 6 | − | 9 | − | + | + | + | + | + | + | + | + | + |
| 19 | + | 5 | + | 9 | + | − | − | − | + | − | − | + | + | + |
| 20 | + | 4 | + | 8 | + | + | + | + | + | + | + | + | + | + |

TABLE II.—*The constancy of the Bacterium lactis acidi and coccus groups in their action upon test substances*—Continued

MICROCOCCUS

| No. of culture. | Production of acid in broth containing— | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sucrose. | | | | | Glycerin. | | | Salicin. | | | Mannit. | | |
| | First test. | Age of culture at time of second test. | Second test. | Age of culture at time of third test. | Third test. | First test. | Second test. | Third test. | First test. | Second test. | Third test. | First test. | Second test. | Third test. |
| | | *Months.* | | *Months.* | | | | | | | | | | |
| 21 | − | 8 | − | 11 | + | − | − | − | − | − | − | − | − | − |
| 22 | − | 10 | + | 14 | − | − | − | + | − | − | − | − | − | − |
| 23 | + | 9 | + | 13 | + | − | − | − | − | − | − | − | − | − |
| 24 | + | 9 | + | 13 | + | + | − | − | − | − | − | − | − | − |
| 25 | + | 6 | + | 9 | + | − | − | − | + | − | − | − | − | − |
| 26 | − | 6 | − | 9 | + | + | − | − | + | + | + | − | − | − |
| 27 | + | 4 | + | 8 | + | + | − | − | + | + | + | − | − | − |

In the *Bacterium lactis acidi* group there is a tendency for those cultures which have a low ability for fermentation to increase that ability when cultivated upon the plain agar slopes. This appears to be a change in the physiological properties of the cultures when grown under artificial conditions, for every variation which occurred in this group was an increased ability to attack the more difficultly fermentable substances. Thus, cultures 2 and 3, which at the time the first test was made fermented only lactose and were classified as variety "a," in later tests fermented also salicin and mannit and would therefore be classified as variety "d." Also, culture 5 acquired the ability to ferment mannit, and culture 6 acquired the ability to ferment salicin.

The Streptococcus cultures all remained constant in their action upon lactose, salicin, and mannit. Cultures 9 and 10 acquired the ability to ferment sucrose, and culture 10 also acquired the property of fermenting glycerin. Culture 19 lost the power of fermenting glycerin. The Micrococcus cultures also showed some inconstancy upon sucrose and glycerin, and one culture, 25, varied in its action on salicin. On the whole, the reactions are sufficiently constant to be considered valuable in the differentiation of cheese organisms.

## CLASSIFICATION OF THE BACTERIUM CASEI GROUP BY BIOCHEMICAL TESTS

The study of a number of cultures of the *Bacterium casei* group isolated from Cheddar cheese showed that there is a great variation in the amount of acidity the different cultures are able to produce in milk. It has been found (Currie, 1911) also that the cultures differ in the rotatory power of their acids. It was evident that different strains of this group were present in cheese, and therefore it was desirable to classify them, in order to study the significance of the various strains in cheese ripening. Accordingly the biochemical tests which were used for the differentiation of the other cheese organisms were adopted also for the classification of this group.

The preliminary study upon the 10 original test substances showed that lactose, salicin, sucrose, mannit, and inulin have some value in the differentiation of this group. The other five test substances were discarded as of too little value to compensate for the time required for their use.

The morphology of this group differentiates it from the other cheese organisms.

Two hundred and forty-nine cultures of the *Bacterium casei* group have been submitted to the tests, which differentiated them into 12 strains, as given in Table III. It was necessary to combine this large number of strains into varieties, in order to make the classification workable in the study of the cheese problem.

TABLE III.—*Types of Bacterium casei as differentiated by biochemical tests*

| Strain No. | Number of cultures studied. | Production of acid in broth containing— | | | | |
|---|---|---|---|---|---|---|
| | | Lactose. | Salicin. | Sucrose. | Mannit. | Inulin. |
| 1 | 5 | − | − | − | − | − |
| 2 | 117 | + | − | − | − | − |
| 3 | 22 | + | + | − | − | − |
| 4 | 12 | + | − | + | − | − |
| 5 | 18 | + | + | + | − | − |
| 6 | 7 | + | − | − | + | − |
| 7 | 14 | + | + | − | + | − |
| 8 | 7 | + | − | + | + | − |
| 9 | 28 | + | + | + | + | − |
| 10 | 1 | + | − | + | − | + |
| 11 | 12 | + | + | + | − | + |
| 12 | 7 | + | + | + | + | + |

Strain 2, which ferments lactose alone and includes almost one-half of the cultures studied, naturally formed one variety. With it was included the five cultures which failed to ferment lactose in broth cultures, although they fermented lactose in milk cultures. The second variety includes all cultures which ferment lactose, and one, two, or three of the test substances—salicin, sucrose, and mannit. A third variety is characterized by its ability to ferment inulin. This variety also ferments lactose and one, two, or three of the substances, salicin, sucrose, and mannit. The three varieties have been designated "a," "b," and "c" in Table IV.

TABLE IV.—*Varieties of Bacterium casei found in cheese*

| Variety No. | Production of acid in broth containing— | | | | |
|---|---|---|---|---|---|
| | Lactose. | Salicin. | Sucrose. | Mannit. | Inulin. |
| a | + | − | − | − | − |
| b | + | ± | ± | ± | − |
| c | + | ± | ± | ± | + |

There is a great variation in the amount of acidity formed in milk by the various cultures of a given strain. In Table V are given the range of maximum percentages of acidity found within the strain. The average percentage of acidity formed by the cultures increases with the ability to break down the more complex test substances. The average percentages for the three varieties are given in Table VI. The acid has been calculated as lactic acid.

TABLE V.—*Range of percentages of acidity formed by the strains of Bacterium casei*

| Strain No. | Number of cultures studied. | Range of maximum percentages of acidity. | Strain No. | Number of cultures studied. | Range of maximum percentages of acidity. |
|---|---|---|---|---|---|
| 1 | 1 | 0.68 | 7 | 12 | 0.91 to 1.97 |
| 2 | 60 | .10 to 1.52 | 8 | 6 | .94 to 1.69 |
| 3 | 8 | .73 to 2.37 | 9 | 14 | 1.38 to 1.80 |
| 4 | 12 | .42 to 1.50 | 10 | 1 | .97 |
| 5 | 16 | .47 to 1.97 | 11 | 6 | .86 to 1.60 |
| 6 | 5 | .85 to 1.35 | 12 | 1 | 1.74 |

TABLE VI.—*Variation in the average percentage of acidity formed by the varieties of Bacterium casei*

| Variety No. | Number of cultures titrated. | Average percentage of acidity. |
|---|---|---|
| a | 60 | 0.75 |
| b | 72 | 1.03 |
| c | 10 | 1.23 |

The statement was made in a previous publication (Hastings, Evans, and Hart, 1912) that the width of the cells of the lactic bacilli in a pure culture appear to be fairly constant, but that in come cultures the individual cells are all slender, whereas in other cultures they are all comparatively thick. Measurements have been made of the width of the cells in many cultures. The averages for the three varieties are given in Table VII.

TABLE VII.—*Variations in the average width of the cells of the varieties of Bacterium casei*

| Variety No. | Number of cultures measured. | Average width of the cells in microns. |
|---|---|---|
| a | 25 | 1.06 |
| b | 22 | .81 |
| c | 3 | .53 |

Thus, it is shown that the width of the cells decreases with their ability to attack the more difficultly fermentable substances and with their ability to produce higher percentages of acidity. The differences in width are so great that with a glance at a microscopic field of a culture containing

cells of either extreme in width, the culture may be classed as a high or a low acid-producing variety.

## DISTRIBUTION OF THE GROUPS AND VARIETIES OF CHEESE ORGANISMS IN NORMAL CHEDDAR CHEESE

Twenty-one raw-milk cheeses have been analyzed bacteriologically and the cultures classified in the manner described above. Some of the cheeses were analyzed only once or a few times. In Table VIII are given the percentages of the different varieties which were found in a series of 16 cheeses. These were the best of a large number of cheeses which had been sent for scoring to the Dairy Department of the University of Wisconsin from cheese factories in different parts of the State. They represent a high quality of Wisconsin cheese.

It will be observed that almost every variety of each of the four groups of cheese organisms is represented in the 16 cheeses in sufficiently large numbers to be influential in the development of flavor. It is not to be supposed that representatives of all of the varieties occurring in a cheese can be isolated each time the cheese is submitted to analysis. Particularly in the case of those cheeses which were analyzed only once, other forms than those shown by the data must have been present. For this reason Table VIII as a whole rather than the data from any individual cheese should be considered in drawing conclusions as to the types of organisms present in normal cheese. The figures of the table indicate that the flora of a normal Cheddar cheese is varied and consists of representatives of each of the four groups of cheese organisms.

TABLE VIII.—*Bacterial content of normal raw-milk cheeses*

| Cheese No. | Age. | Bacteria per gram (plate cultures). | Variety of Bacterium lactis acidi. | | | Variety of Bacterium casei. | | | Variety of streptococci. | | | | Variety of micrococci. | | | Bacteria per gram (dilution cultures). | Variety of Bacterium casei. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | a | b | d | a | b | c | a | b | c | d | a | b | c | | a | b |
| | *Days.* | | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | | *P.c.* | *P.c.* |
| 33..... | 30 | 35,000,000 | .... | .... | 100 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 100,000,000 | a+ | .... |
| 35..... | 30 | 8,000,000 | .... | 63 | .... | 25 | .... | .... | .... | .... | .... | 12 | .... | .... | .... | 10,000,000 | .... | a+ |
| 44..... | 32 | 4,000,000 | 25 | .... | .... | .... | .... | 12 | .... | .... | .... | 25 | .... | 38 | .... | 10,000,000 | .... | a+ |
| 36..... | 28 | 10,500,000 | 40 | .... | .... | 10 | 20 | .... | .... | 30 | .... | .... | .... | .... | .... | 1,000,000,000 | .... | a+ |
| 49..... | 32 | 3,200,000 | .... | .... | 71 | 29 | .... | .... | .... | .... | .... | .... | .... | .... | .... | 10,000,000 | .... | a+ |
| 38..... | 48 | 2,000,000 | 12 | .... | 13 | 12 | .... | .... | .... | 25 | 13 | 12 | .... | 13 | .... | 1,000,000 | .... | .... |
| 45..... | 38 | 55,000,000 | 67 | .... | .... | .... | 22 | .... | .... | .... | .... | 11 | .... | .... | .... | 100,000,000 | .... | a+ |
| 50..... | 24 | 7,000,000 | 30 | .... | 50 | .... | .... | .... | 10 | .... | 10 | .... | .... | .... | .... | 10,000,000 | .... | .... |
| 53..... | 30 | 8,000,000 | 70 | .... | .... | .... | .... | .... | .... | .... | 30 | .... | .... | .... | .... | 10,000,000 | .... | a+ |
| 63..... | 30 | 3,500,000 | 10 | 20 | 50 | .... | 10 | .... | .... | 10 | .... | .... | .... | .... | .... | 10,000,000 | .... | a+ |
| 5..... | 45 | 18,500,000 | .... | .... | .... | .... | 90 | .... | .... | .... | 10 | .... | .... | .... | .... | 100,000,000 | .... | a+ |
| | 52 | 36,000,000 | 11 | .... | .... | .... | 67 | .... | 11 | .... | 11 | .... | .... | .... | .... | 10,000,000 | .... | a+ |
| | 68 | 11,000,000 | 20 | .... | .... | .... | 80 | .... | .... | .... | .... | .... | .... | .... | .... | 10,000,000 | .... | a+ |
| 13..... | 37 | 28,000,000 | 50 | .... | .... | .... | 33 | .... | .... | .... | .... | .... | 17 | .... | .... | 100,000,000 | a+ | .... |
| | 43 | 25,000,000 | 63 | .... | .... | 13 | .... | .... | 12 | .... | .... | .... | 12 | .... | .... | 100,000,000 | .... | .... |
| | 59 | 3,200,000 | .... | .... | 30 | 10 | 48 | .... | .... | 10 | .... | 10 | .... | .... | .... | 1,000,000 | .... | .... |
| 16..... | 39 | 10,000,000 | 14 | .... | .... | 58 | .... | .... | .... | .... | .... | .... | 28 | .... | .... | 10,000,000 | .... | a+ |
| | 46 | 5,000,000 | 14 | .... | .... | .... | 86 | .... | .... | .... | .... | .... | .... | .... | .... | 10,000,000 | a+ | .... |
| | 62 | 4,500,000 | .... | .... | .... | .... | 100 | .... | .... | .... | .... | .... | .... | .... | .... | 10,000,000 | .... | .... |
| 17..... | 18 | 12,000,000 | 90 | .... | .... | .... | .... | .... | .... | .... | .... | 10 | .... | .... | .... | 10,000,000 | .... | a+ |
| | 25 | 15,000,000 | 50 | .... | .... | .... | 10 | .... | .... | .... | .... | .... | 40 | .... | .... | 100,000,000 | .... | .... |
| | 41 | 11,500,000 | 10 | .... | .... | 80 | 10 | .... | .... | .... | .... | .... | .... | .... | .... | 1,000,000 | .... | .... |
| 130.... | 16 | 600,000,000 | .... | .... | 17 | 83 | .... | .... | .... | .... | .... | .... | .... | .... | .... | 100,000,000 | .... | .... |
| | 32 | 125,000,000 | .... | 78 | .... | .... | .... | .... | .... | 11 | .... | 11 | .... | .... | .... | 1,000,000,000 | .... | .... |
| 131.... | 14 | 38,000,000 | .... | 76 | .... | .... | 11 | .... | .... | .... | .... | .... | 11 | .... | 2 | 100,000,000 | .... | .... |
| | 30 | 120,000,000 | .... | 11 | .... | 88 | .... | .... | .... | .... | .... | .... | 1 | .... | .... | 100,000,000 | .... | .... |

a +=Found present.

A more comprehensive study was made of raw-milk cheese No. 17 R. Analyses were made during the making and at intervals throughout the ripening period until the cheese was 8 months old. The results of this study are given in Table IX.

TABLE IX.—*Bacterial content of raw-milk cheese No. 17 R*

| Age. | Bacteria per gram (plate cultures). | Variety of Bacterium lactis acidi. | | | | Variety of Bacterium casei. | | Variety of streptococci. | | | Variety of micrococci. | | Bacteria per gram (dilution cultures). | Variety of Bacterium lactis acidi. | | | Variety of Bacterium casei. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | a | b | c | d | a | b | b | c | d | a | b | | a | b | d | a | b |
| *Days.* | | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | *P.c.* | | *P.c.* | *P.c.* | *P. c.* | *P.c.* | *P. c.* |
| Curd. | 100,000,000 | .... | 30.0 | .... | 70.0 | .... | .... | .... | .... | .... | .... | .... | 100,000,000 | .... | .... | 99.999 | .... | 0.001 |
| 4..... | 70,000,000 | 10.0 | .... | .... | 40.0 | .... | .... | 50.0 | .... | .... | .... | .... | 100,000,000 | .... | .... | ..... | .... | ..... |
| 8..... | 900,000,000 | .... | 88.8 | .... | .... | .... | .... | .... | 11.2 | .... | .... | .... | 1,000,000,000 | .... | .... | 9.99 | .... | .01 |
| 15.... | 120,000,000 | .... | .... | 90.0 | .... | .... | 10 | .... | .... | .... | .... | .... | 1,000,000,000 | .... | 99.0 | ..... | .... | 1.00 |
| 22.... | 240,000,000 | .... | 87.5 | .... | .... | 12.5 | .... | .... | .... | .... | .... | .... | 100,000,000 | 99.0 | .... | ..... | 1.0 | ..... |
| 29.... | 280,000,000 | 66.3 | .... | .... | 22.2 | .... | .... | .... | .... | 11.2 | 0.3 | .... | 1,000,000,000 | 99.0 | .... | ..... | 1.0 | ..... |
| 36.... | 400,000,000 | .... | 10.0 | .... | 80.0 | .... | .... | .... | .... | .... | 10.0 | .... | 100,000,000 | .... | .... | 90.90 | 9.1 | ..... |
| 43.... | 160,000,000 | 43.0 | .... | 56.3 | .... | .... | .... | .... | .1 | .2 | .1 | 0.3 | 100,000,000 | 90.9 | .... | ..... | 9.1 | ..... |
| 50.... | 200,000,000 | .... | 70.0 | .... | .... | .... | .... | 17.5 | .... | .... | 12.5 | .... | 100,000,000 | 90.9 | .... | ..... | 9.1 | ..... |
| 57.... | 100,000,000 | .... | .... | .... | 76.5 | .... | .... | .... | .... | 20.0 | 3.5 | .... | ..... | .... | .... | ..... | ..... | ..... |
| 64.... | 80,000,000 | .... | 88.8 | .... | .... | .... | .... | .... | .... | 10.0 | 1.2 | .... | 100,000,000 | .... | 90.9 | ..... | 9.1 | ..... |
| 71.... | 100,000,000 | 35.1 | .... | .... | 41.7 | 20.0 | .... | .... | .... | .... | 3.2 | .... | 100,000,000 | .... | 90.9 | ..... | 9.1 | ..... |
| 89.... | 32,000,000 | 86.9 | .... | .... | .... | .... | .... | 10.0 | .... | .... | 3.1 | .... | 100,000,000 | 99.0 | .... | ..... | 1.0 | ..... |
| 172... | 7,000,000 | .... | 22.0 | .... | 67.0 | .... | .... | .... | .... | .... | 11.0 | .... | 100,000,000 | .... | .... | 90.9 | 9.1 | ..... |
| 245... | 12,000,000 | .... | .... | .... | .... | 20.0 | 80 | .... | .... | .... | .... | .... | 100,000,000 | .... | .... | 50.0 | 50.0 | ..... |

Where very small percentages appear in the columns of the coccus groups the figures were obtained by plating upon an agar made with cheese extract. Upon this medium the lactic-acid groups of organisms form very small colonies, but the cocci, particularly the micrococci, develop large colonies and can be easily differentiated and isolated from plates which are very thickly seeded.

Cheese No. 17 R was a good Cheddar cheese, with the characteristic flavor well developed when 1 month old. The cheese remained in good condition until the last analysis, with an increasing sharpness of taste which is common to well-matured cheese. The flora is considered typical of a good Cheddar cheese. The data confirm those presented in Table VIII. Twelve of the fifteen varieties of cheese organisms representing the four groups were isolated from this cheese. The data presented in Tables VIII and IX show that besides the *Bacterium lactis acidi* group, which has long been known to be important in cheese ripening, and the *B. casei* group, which was shown (Hastings, Evans, and Hart, 1912) to be constantly present in cheese in numbers almost as great as the former group. Coccus forms are also constantly present, and although they are fewer in number than the lactic-acid-forming groups they occur in sufficient numbers to affect flavor development.

Other evidence of the constant presence of several varieties of bacteria in the cheese flora is found in the gradual decline in acid production, as the dilutions increase, in milk inoculated with the cheese emulsion. In the present study of cheese flora, several hundreds of series of titrations

have been made of the milk cultures which have been mentioned as a part of the routine method of cheese analysis. There is in nearly every series a general decline as the emulsion used for the inoculation becomes more and more dilute. The two series presented in Table X illustrate the point. The titrations were made after 28 days incubation at 35° C. and the acidity calculated as lactic acid.

TABLE X.—*Decline in acidity with increasing dilutions of cheese emulsion used for inoculation of milk cultures*

| Cheese No. 312 C. | | Cheese No. 309 C. | |
|---|---|---|---|
| Dilution. | Percentage of acidity. | Dilution. | Percentage of acidity. |
| 1:100,000 | 1.60 | 1:10,000 | 1.54 |
| 1:1,000,000 | 1.55 | 1:100,000 | 1.45 |
| 1:10,000,000 | 1.22 | 1:1,000,000 | 1.50 |
| 1:100,000,000 | .78 | 1:10,000,000 | 1.18 |
| 1:1,000,000,000 | .67 | 1:100,000,000 | 1.00 |
| 1:10,000,000,000 | (a) | 1:1,000,000,000 | (a) |

[a] No growth.

The lower percentage of acidity with every tenfold dilution indicates that in each dilution there were present certain varieties of organisms in such small proportions that they did not occur in the next higher dilution. Out of 345 series of titrations the decline was without a break in 56 per cent of the cases; in 37 per cent of the cases there was a general decline, but with one or more breaks; in only 7 per cent of the cases was this decline not apparent.

## THE VARIATION IN CHEESE FLORA

It is evident from Tables VIII and IX that the normal flora of Cheddar cheese is varied, with varieties of all four groups of cheese organisms in numbers ranging from hundreds of thousands to billions per gram of cheese. The flora in two equally good cheeses will contain each of the four groups of cheese organisms, but will differ as to the varieties present. There will also be a variation in the proportion of organisms of any single group. It does not appear that it is essential to good flavor to have any single variety present in very high proportions. One cheese may contain a given variety in high proportions, whereas another cheese may have this variety in such low proportions that it does not make its appearance in a bacteriological analysis. To illustrate: Streptococcus "b," which formed 30 per cent of the flora of cheese No. 36 (Table VIII), was isolated from only 6 of the 17 cheeses whose flora are presented in Tables VIII and IX.

This variation of flora, with a constancy to the general type of growth, is to be expected under any ecological conditions determined by natural

circumstances, for the ripening of Cheddar cheese must be regarded as a spontaneous fermentation, limited to certain groups of bacteria by the conditions obtaining in the cheese mass and subject to variation within the groups by accident of inoculation before or during the cheese making, or by slight variations in the raw material or in the method of manufacture, which enable one variety or the other to gain an ascendancy.

## SYMBIOTIC RELATION OF CHEESE ORGANISMS

The complex chemical changes taking place in a mixed culture of bacteria have usually been regarded as the result of the combined activity of the individual properties of each of the several species when grown alone, with these properties accelerated in some cases by the associated action.

Among the many cultures of cheese organisms inoculated into the carbohydrate media, it was observed that when by accident the culture was contaminated with other cheese organisms through failure of a pure isolation, there appeared to be a high ability to attack the more complex substances. Accordingly many inoculations of cheese cultures were made into the test media in various combinations, to determine whether associative action would give new properties to the cultures concerned. In this experiment the test for acid production was the reddening of litmus paper. In many cases acid was produced from a given substance by the associated action, when neither culture working alone would give such a reaction. A few examples are given in Table XI to show the results of these inoculations.

TABLE XI.—*The effect of the associative action of cheese organisms in breaking down test substances*

| Organism. | Production of acid in broth containing— | | | | | |
|---|---|---|---|---|---|---|
| | Lactose. | Salicin. | Sucrose. | Glycerin. | Mannit. | Inulin. |
| Bacterium lactis acidi, a | + | − | − | − | − | ..... |
| Bacterium casei, a | + | − | − | ..... | − | − |
| Bacterium casei, b | + | + | − | ..... | + | − |
| Streptococcus, b | + | − | − | − | − | ..... |
| Streptococcus, c | + | + | + | − | − | ..... |
| Micrococcus, b | + | − | − | − | − | ..... |
| Bacterium lactis acidi, a; Bacterium casei, a | + | − | + | − | − | − |
| Bacterium lactis acidi, a; Bacterium casei, b | + | + | + | − | + | − |
| Bacterium lactis acidi, a; Streptococcus, b | + | + | + | − | − | − |
| Bacterium lactis acidi, a; Streptococcus, c | + | + | + | + | − | − |
| Bacterium casei, a; Bacterium casei, b | + | + | + | − | + | − |
| Bacterium casei, a; Streptococcus, b | + | − | + | − | − | − |
| Bacterium casei, a; Streptococcus, c | + | + | + | + | + | − |
| Bacterium casei, a; Micrococcus, b | + | + | + | − | − | − |

Further inoculations were made into broth containing the test substances to determine the quantitative effect of this associative action. The results of some of these inoculations are given in Table XII, in which the acidity is expressed as lactic acid.

TABLE XII.—*The effect of the associative action of cheese organisms in the production of acid*

| Organism. | Medium. | | | | | |
|---|---|---|---|---|---|---|
| | Salicin. | | Sucrose. | | Mannit. | |
| | Individual action. | Associative action. | Individual action. | Associative action. | Individual action. | Associative action. |
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Bacterium lactis acidi, a | 0.00 | 0.39 | 0.00 | 0.00 | 0.00 | 0.00 |
| Bacterium casei, a | .00 | | .00 | | .00 | |
| Bacterium lactis acidi, a | .00 | .32 | .00 | .00 | .00 | .00 |
| Streptococcus, b | .00 | | .00 | | .00 | |
| Bacterium lactis acidi, b | .00 | .37 | .00 | .28 | .00 | .00 |
| Bacterium casei, a | .30 | | .00 | | .00 | |
| Bacterium lactis acidi, b | .00 | .32 | .00 | .51 | .00 | .35 |
| Micrococcus, c | .39 | | .44 | | .00 | |
| Bacterium lactis acidi, d | .00 | .00 | .00 | .17 | .00 | .28 |
| Bacterium casei, a | .00 | | .00 | | .00 | |
| Bacterium lactis acidi, d | .00 | .21 | .00 | ..... | .00 | .31 |
| Micrococcus, b | .00 | | .00 | | .33 | |

The data show that considerable quantities of acid can be formed in mixed cultures even though the individual cultures are unable to ferment the substance in question. In the *Bacterium lactis acidi* group, 13 positive reactions were obtained and determined by titration in cultures in which a member of this group was mixed with a representative of one of the other three groups of cheese organisms, while 23 cases were negative. In the entire 36 tests none of the organisms was able to ferment the substance used when working alone. The same symbiotic relations were exhibited by the *B. casei* group when a member of this group was grown together with a representative of one of the other groups of cheese organisms or with another variety of the same group. (See Table XI.) Thus, it appears not only that the influence upon each other of the cheese organisms is beneficial in enhancing the individual powers in many cases but also that the symbiosis enables the cheese organisms to attack other food substances than any one species working alone would be able to utilize.

The fact that the normal flora of Cheddar cheese consists of a number of varieties of the four groups of cheese organisms and that these exert upon one another a decided influence in their chemical activities is most important in considering the cheese-ripening problem.

## BACTERIAL FLORA OF PASTEURIZED MILK CHEESE

The influence of this varied flora upon the production of flavor is emphasized in a study of the flora of pasteurized-milk cheese. During the pasteurization approximately 99 per cent of the bacteria of the milk are killed (Sammis and Bruhn, 1912); then there are added, in the form of a starter, organisms belonging to the *Bacterium lactis acidi* group. But instead of ripening the milk until the desired acidity for cheese making is obtained—the usual procedure in the making of raw-milk cheese—a weak solution of hydrochloric acid is added to make up the required acidity. This is necessary in preparing pasteurized milk for cheese making.

It is evident that the initial bacterial flora of the cheese made from pasteurized milk must differ from the flora of a raw-milk cheese of the same age, for in the latter all of the types of organisms making up the varied flora of milk have had an opportunity to develop without any other restraint than that of their symbiotic relations with the associated types.

In studying the flora of cheese in respect to its influence in flavor production many bacterial analyses have been made of pasteurized-milk cheese in every stage of the ripening process. The cultures isolated have been studied in the same manner as those from raw-milk cheese and have been classified accordingly. In Tables XIV and XV are presented the results of the analyses of two pasteurized-milk cheeses, Nos. 20 and 21, to which pure-culture starters were added. To cheese No. 20 (Table XIII), there was added 0.75 per cent of a pure-milk culture of *Bacterium lactis acidi*, b. In striking contrast to the varied flora of raw-milk cheese, as presented in Tables VIII and IX, it will be observed that the bacterial flora of this cheese consisted almost entirely of the one variety of organism, *B. lactis acidi*, b, which was added to the milk for a starter. Only once was another variety of this group isolated.

TABLE XIII.—*Bacterial content of pasteurized-milk cheese No. 20 to which was added 0.75 per cent of a culture of Bacterium lactis acidi, b*

| Age. | Plate cultures. | | | | | | Dilution cultures. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial content. | Variety of Bacterium lactis acidi. | | Variety of streptococci. | Variety of micrococci. | | Contamination. | Bacterial content. | Variety of Bacterium lactis acidi. | Variety of Bacterium casei. | |
| | | a | b | d | a | b | | | b | a | b |
| *Days or state.* | | P. ct. | P. ct. | P. ct. | P. ct. | P. ct. | P. ct. | | P. ct. | P. ct. | P. ct. |
| Milk.. | 9,600,000 | ..... | 99.70 | ..... | ..... | ..... | 0.30 | 100,000,000 | ..... | ..... | 0.0001 |
| Curd . | 20,000,000 | ..... | 98.00 | ..... | ..... | ..... | 2.00 | 100,000,000 | 99.9999 | 0.0001 | ..... |
| 6.... | 450,000,000 | ..... | 100.00 | ..... | ..... | ..... | ..... | 1,000,000,000 | ..... | ..... | ..... |
| 14.... | 175,000,000 | ..... | 96.80 | ..... | 0.2 | ..... | 3.00 | 100,000,000 | 100.00 | ..... | ..... |
| 22.... | 175,000,000 | ..... | 98.30 | ..... | ..... | ..... | 1.70 | 100,000,000 | 100.00 | ..... | ..... |
| 30.... | 400,000,000 | ..... | 95.75 | ..... | ..... | ..... | 4.25 | 10,000,000,000 | ..... | ..... | ..... |
| 38.... | 300,000,000 | 28.2 | 70.50 | ..... | ..... | ..... | 1.30 | 1,000,000,000 | ..... | ..... | ..... |
| 47.... | 1,650,000,000 | ..... | 100.00 | ..... | ..... | ..... | ..... | 1,000,000,000 | 99.00 | 1.0000 | ..... |
| 58.... | 65,000,000 | ..... | 99.77 | 0.04 | ..... | 0.19 | ..... | ..... | ..... | ..... | ..... |
| 72.... | 170,000,000 | ..... | 100.00 | ..... | ..... | ..... | ..... | 100,000,000 | 90.90 | ..... | 9.1000 |

One variety of the Streptococcus group was isolated once, and two varieties of the Micrococcus were isolated, each only once, in small percentages. The small percentages of cocci were isolated from cheese-agar plates in the same manner as was followed in the analysis of the raw-milk cheese presented in Table IX. Cheese No. 20 was quite badly contaminated with a liquefying coccus which infected each of the three vats of milk made up separately into cheese. The contamination is given in a separate column. The data from the dilution cultures show *Bacterium lactis acidi*, b, as the predominating organism of the cheese at every examination. The *B. casei* group developed slowly in this cheese. It was present in exceedingly small percentage during the first few days. Unfortunately the dilutions were made too high, so that this group did not appear in the dilution cultures in the analyses made between the sixth and forty-seventh days. At the forty-seventh and seventy-second days there were present 1,000,000 bacteria of this group per gram of cheese.

TABLE XIV.—*Bacterial content of pasteurized-milk cheese No. 21 to which was added 0.75 per cent of a culture of Bacterium lactis acidi, d*

| Age. | Plate cultures. | | | | Dilution cultures. | | | |
|---|---|---|---|---|---|---|---|---|
| | Bacteria content. | Variety of B. lactis acidi, d. | Variety of micrococci. | | Bacterial content. | Variety of B. lactis acidi, d. | Variety of Bact. casei. | |
| | | | a | b | | | a | b |
| *Days or state.* | | *Per cent.* | *Per ct.* | *Per ct.* | | *Per ct.* | *Per ct.* | *Per ct.* |
| Milk | | | | | 10,000,000 | 100.00 | | |
| Curd | 250,000,000 | 100.00 | | | 100,000,000 | | | |
| 5 | 270,000,000 | 100.00 | | | 100,000,000 | 99.999 | 0.001 | |
| 13 | 1,120,000,000 | 100.00 | | | 1,000,000,000 | 100.00 | | |
| 21 | 1,200,000,000 | 100.00 | | | 1,000,000,000 | | | |
| 29 | 250,000,000 | 100.00 | | | 1,000,000,000 | 100.00 | | |
| 37 | 1,400,000,000 | 99.99 | 0.01 | | 1,000,000,000 | | | |
| 46 | 1,200,000,000 | 99.99 | .01 | | 1,000,000,000 | 99.90 | .100 | |
| 57 | 1,000,000,000 | 99.94 | .02 | 0.04 | | | | |
| 71 | 1,480,000,000 | 100.00 | | | 1,000,000,000 | 99.90 | | 0.1 |

Cheese No. 21 (see Table XIV) received three-fourths per cent of *Bacterium lactis acidi*, d, as a starter. A study of the pure cultures isolated from this cheese shows that this variety composed practically 100 per cent of the cheese organisms, as determined by plate cultures. No other variety of *Bacterium lactis acidi* and no variety of the Streptococcus group appeared. Small percentages of micrococci were isolated on the thirty-seventh, forty-sixth, and fifty-seventh days. The pure cultures isolated from the dilution flasks showed only *B. lactis acidi*, d, and a slowly increasing number of *B. casei*, which reached 1,000,000 of these bacteria per gram of cheese on the forty-sixth day.

The data in Tables XIII and XIV illustrate well the facts which are demonstrated by a study of the results of many analyses of 14 pasteurized-milk cheeses. In some of these cheeses a mixture of several

pure cultures was used as a starter. Usually every type which was added to the cheese was isolated in considerable percentages in some one or more of the analyses. Thus, it has been shown that the flora of pasteurized-milk cheese consists almost entirely of the organisms which are introduced in the starter, with a small percentage of micrococci and steadily increasing numbers of *Bacterium casei*.

## RELATION OF THE VARIOUS TYPES OF CHEESE ORGANISMS TO FLAVOR PRODUCTION

Since it has been shown that in pasteurized-milk cheese the bacterial content is practically limited to the varieties which are added during the making, together with small percentages of micrococci, and a development of the *Bacterium casei* group similar to that in a raw-milk cheese, although it is usually more or less retarded, it is possible to gain some knowledge of the influence of the different varieties in pure culture upon flavor production.

A normal raw-milk cheese of the Cheddar type will, after a few days' ripening, begin to develop a delicate flavor which is characteristic of this type of cheese. This flavor becomes intensified as the cheese matures, and after ripening for a number of weeks, depending upon the temperature at which the cheese is kept, it acquires a pungent taste which also intensifies with continued ripening.

A pasteurized-milk cheese made with a commercial starter will never develop the Cheddar flavor which characterizes a young raw-milk cheese of this type, but it does develop an acid flavor which is pleasant to the taste of many people. If the cheese is kept for several months, the acid flavor disappears and the biting taste common to well-matured raw-milk cheese becomes the characteristic flavor.

## THE RÔLE OF BACTERIUM CASEI IN FLAVOR PRODUCTION

The floras of the two types of cheese, which differ essentially during the first few weeks of ripening, become more and more alike as the cheese matures. As shown in Table XV, *Bacterium casei* develops in pasteurized-milk cheese in as large numbers as in raw-milk cheese. It is reasonable, therefore, to ascribe to this group of organisms the development of the pungent flavor common to the two types of well-matured cheese.

In Table XV are given the numbers of *Bacterium casei* in a raw-milk and in a pasteurized-milk cheese made the same day from the same lot of milk. Until the forty-second day the raw-milk cheese contains about 10 times as many of the *B. casei* as the pasteurized-milk cheese, but after the forty-second day the cheeses contain about equal numbers of these organisms.

Cheeses Nos. 307 C and 307 illustrate the *Bacterium casei* content found in 12 similar cheeses—6 of the raw milk and 6 of the pasteurized milk—

made on successive days. The two cheeses selected were among the best flavored of the lot and are typical cheeses of the raw-milk and pasteurized-milk types. The Cheddar flavor was already developing in the raw-milk cheese when first examined for flavor at 18 days. This flavor increased

TABLE XV.—*The correlation of the development of the Bacterium casei group and the pungent taste in raw-milk and pasteurized-milk cheese*

| Age. | Cheese No. 307 C (raw milk). | | Cheese No. 307 (pasteurized milk). | |
|---|---|---|---|---|
| | Number of individuals of Bacterium casei per gram of cheese. | Flavor. | Number of individuals of Bacterium casei per gram of cheese. | Flavor. |
| *Days.* | | | | |
| 2 | 10, 000 | ..... | ..... | |
| 3 | 10, 000 | ..... | 10, 000 | |
| 4 | 100, 000 | ..... | 10, 000 | |
| 5 | 100, 000 | ..... | 10, 000 | |
| 7 | 10, 000, 000 | ..... | 1, 000, 000 | |
| 9 | 100, 000, 000 | ..... | 1, 000, 000 | |
| 11 | 1, 000, 000 | ..... | 1, 000, 000 | |
| 14 | 10, 000, 000 | ..... | 10, 000, 000 | |
| 18 | 10, 000, 000 | Cheddar, developing... | 1, 000, 000 | Cottage-cheese. |
| 21 | 10, 000, 000 | ..... | 100, 000 | |
| 29 | 100, 000, 000 | ..... | 10, 000, 000 | |
| 36 | 100, 000, 000 | ..... | 10, 000, 000 | |
| 42 | 10, 000, 000 | ..... | 10, 000, 000 | |
| 49 | 10, 000, 000 | Strong Cheddar..... | ..... | Only a sour taste. |
| 56 | 10, 000, 000 | ..... | 1, 000, 000 | Do. |
| 71 | 10, 000, 000 | Strong Cheddar..... | 100, 000, 000 | Clean, sour taste; no Cheddar. |
| 98 | 10, 000, 000 | Strong Cheddar; getting sharp. | 10, 000, 000 | Do. |
| 108 | 10, 000, 000 | ..... | 100, 000, 000 | Do. |
| 150 | 1, 000, 000 | Strong Cheddar; sharp.. | 10, 000, 000 | Do. |
| 218 | 10, 000, 000 | Extremely sharp..... | 1, 000, 000 | Acid flavor has disappeared. Mild sharpness. |
| 311 | ..... | Intensely sharp..... | ..... | Good cheese. Sharp. |

in strength until the ninety-eighth day, when the pungent taste became evident. As the cheese aged, the sharpness became more and more intense. In the pasteurized-milk cheese no other flavor than a sour taste was apparent until the cheese was about 7 months old, when it possessed a mild, sharp taste.

Jensen (1904, p. 356) has shown that the *Bacterium casei* group is active in breaking down the casein of milk to which calcium carbonate is added and maintains that the casein is not peptonized, but is split directly into monoamino acids.

Van Slyke and Hart (1903) have shown that there is a constantly increasing percentage of monoamino acids in ripening Cheddar cheese. At three months more than one-third of the water-soluble nitrogenous compounds is in this form, and later a much larger percentage. The evidence seems to point to the fact that in raw-milk Cheddar cheese and

in the pasteurized-milk cheese the *Bacterium casei* group common to both of these types of cheese is responsible for the biting taste which is characteristic of the well-ripened cheese.

Jensen has explained the action of the *Bacterium casei* group in producing amino acids as due to an "endoerepsin" set free by the dead cells rather than to the activity of the living bacteria, because he found the greatest amount of amino acids formed after the bacteria were for the most part dead (Jensen, 1912).

Whatever may be the condition in the Emmenthaler cheese which Jensen studied, this explanation for the activity of the *Bacterium casei* group in Cheddar cheese is not necessary. It appears to be certain that the living bacteria were active throughout the ripening period in this type of cheese. Cheese No. 17 R (Table IX) and cheese No. 307 C (Table XV) both demonstrated this fact. In cheese No. 17 R there were 10,000,000 living bacteria of the *B. casei* group per gram of cheese when 8 months old; cheese No. 307 C contained a similar number at 7 months. These bacteria did not appear to be living in a latent condition, for all three varieties of *B. casei* readily grew upon an agar made with only an extract of the cheese for a food substance. Every time an analysis was made of cheese No. 17 R such an agar was prepared on the day the analysis was made, using for the cheese agar a part of the plug which served for bacteriological analysis. Thus, the cheese organisms were submitted for development upon the same food substance which served them in the cheese itself.

The result of the bacterial count upon this cheese agar was only slightly smaller than the total count upon the casein agar. Every variety of cheese organisms grew upon this medium. On the eighty-ninth day, when 32,000,000 organisms per gram of cheese developed colonies on the casein agar and of the 10 colonies isolated none belonged to the *Bacterium casei* group, 20,000,000 colonies developed upon the cheese agar, of which 20 per cent, or 4,000,000 bacteria per gram, belonged to the *B. casei* group. The flask dilutions on that same date showed 1,000,000 bacteria of this group per gram of cheese. Thus, it is shown that several millions of living organisms of the *B. casei* group are present in a normal Cheddar cheese 3 months of age, and the cheese itself provides suitable food for development. It is most probable that under these conditions the living bacteria are active in the cheese.

## INFLUENCE OF BACTERIUM CASEI IN STARTERS FOR PASTEURIZED-MILK CHEESE

Many experimental pasteurized-milk cheeses have been made with pure-culture starters, to determine their influence upon the production of flavor.

In the first series some variety of *Bacterium casei* was added to a number of the cheeses, together with the *B. lactis acidi*. When variety "a" was added, there was a tendency for the cheese to become "acid injured"—strongly acid, friable, and opaque. The use of variety "b" as a starter

was more likely to bring about this condition. When variety "c" was used, the cheese was almost certain to be ruined by the acid before it was a month old. This variety was isolated only once from the 21 normal raw-milk cheeses which have been studied with reference to the varieties; and then there were present less than 500,000 bacteria per gram of cheese. *B. casei*, c, has been isolated, however, from three raw-milk cheeses which had a sour taste rather than the Cheddar flavor. Therefore this variety can not be regarded as a necessary organism in normal ripening. It is likely that it never occurs in large numbers in the young cheese without causing injury. *B. casei*, varieties "a" and "b," are about equally distributed in normal Cheddar cheese, where they usually occur together and perform an active part in the ripening changes. It has been noted that the *B. casei* groups develop gradually in the pasteurized-milk cheese, as they do in the raw-milk cheese, although usually more slowly. The introduction of this group as a starter, however, resulting in abnormally large numbers of *B. casei* in the early ripening period, is detrimental to the cheese.

In subsequent series of experiments with pasteurized-milk cheese this group of organisms was never added to the starter.

### INFLUENCE OF BACTERIUM LACTIS ACIDI UPON FLAVOR PRODUCTION

When *Bacterium lactis acidi*, a or b, is used for a starter in pure culture in pasteurized-milk cheese, an acid taste is produced which is characteristic of the ordinary pasteurized-milk cheese made with the use of a commercial starter. No suggestion of a Cheddar flavor is ever obtained. If *B. lactis acidi*, d, is added to the milk, there is almost always produced a peculiar flavor, which, as it intensifies with continued ripening, becomes decidedly bitter. Out of 14 experimental cheeses in which 0.75 per cent, the ordinary quantity used for a starter, of a pure culture of this variety was used or in which a mixture of several pure cultures was used with *B. lactis acidi*, d, in large proportions, the unpleasant flavor has developed some time between the fourth and fourteenth weeks. Usually the cheese becomes bitter by the time it is 2 months old. In only one instance has the bitterness failed to develop before the fourteenth week. The figures in Tables VIII and IX show that *B. lactis acidi*, d, was isolated from normal raw-milk cheese with sufficient frequency that it may be concluded that it is always present in this type of cheese in large numbers. It no doubt contributes to the characteristic Cheddar flavor under the conditions obtaining in a normal cheese. But it is certain that a large amount of this variety is not suitable for use in the starter for pasteurized-milk cheese.

### INFLUENCE OF THE COCCUS GROUPS IN THE PRODUCTION OF FLAVOR

From the frequency of occurrence of all four varieties of the streptococci in normal Cheddar cheese in percentages ranging as high as 50, as shown in Tables VIII and IX, it is most certain that this group is

active in the ripening changes. The by-products of this group of organisms are entirely different from those of the *Bacterium lactis acidi* group. Instead of a large quantity of lactic acid, with small quantities of other acids, as formed by the latter group, the streptococci produce no lactic acid, but produce large amounts of acetic acid, with smaller percentages of propionic, butyric, and caproic acids (Hart, Hastings, Flint, and Evans, 1914). The streptococci were also shown to produce small amounts of ammonia, a by-product not found in *B. lactis acidi* cultures. It is therefore to be expected that the large numbers of streptococci present in Cheddar cheese have a decided influence upon flavor development. The influence of individual cultures of this group in the ripening of pasteurized-milk cheese will be discussed later.

Bacteria of the Micrococcus group were isolated from normal Cheddar cheese in percentages as high as 40. It does not appear that this group of organisms is of primal importance in the production of Cheddar flavor, however, for they have been found commonly in pasteurized-milk cheese in numbers comparable with those found in the raw-milk cheese with well-developed flavor. When added to the cheese in large percentages of the starter, a bitterness is always produced within a few weeks.

## EXPERIMENTS IN THE PRODUCTION OF DESIRABLE FLAVORS IN PASTEURIZED-MILK CHEESE

The study of the bacterial content of normal Cheddar cheese has demonstrated the fact that the flora is varied and is made up of several varieties of all four groups of cheese organisms. Therefore, in the attempt to prepare starters which might develop Cheddar flavors in the pasteurized-milk cheese it appeared reasonable to mix pure cultures together for the cheese inoculation, choosing the varieties most frequently found in Cheddar cheese with the well-developed flavor. Many experimental cheeses have been made with the use of such starters, the mixtures being made up of from two to nine pure cultures in various combinations and varying percentages, in order to determine which of the varieties in addition to *Bacterium lactis acidi* might improve the acid flavor of the pasteurized-milk cheese.

The difficulties in preparing a starter which will reinstate in pasteurized milk a flora which will simulate raw milk well ripened for cheese making are apparent, even though the relative percentages of each group and variety were better known.

Out of 20 experimental cheeses which were inoculated with *Bacterium lactis acidi*, a or b, together with one of the other varieties of this group or some variety of the coccus groups or with some mixture of these pure cultures, there was usually obtained a better flavor in the young cheese than in the control cheese inoculated with a pure culture of *B. lactis acidi*, a or b, alone. But by the time the cheese was well ripened a

bitterness had usually developed. It was observed that, as in the *B. lactis acidi* group, the coccus cultures with a high ability to ferment the more complex test substances were likely to produce bitterness when inoculated in large percentages. The fact that some of these cultures with high fermenting ability were included in almost all of the mixtures is thought to account for the development of bitterness in this series of experimental cheeses. Nevertheless these organisms may participate in the production of a good Cheddar flavor under the conditions for their development in the raw-milk cheese. In only one cheese of this series was a semblance of a Cheddar flavor obtained. This cheese was inoculated with the following mixture: *B. lactis acidi*, b, 42 per cent; *B. lactis acidi*, d, 48 per cent; Streptococcus, c, and Micrococcus, b, each 5 per cent. At three months there was an unmistakable resemblance to a Cheddar flavor. At four months the cheese scored as high as 94.6 per cent when examined by experts. Thus, the Cheddar flavor was obtained with this combination of cultures, most of which produced bitterness when used individually.

It was observed that several cheeses of this series, to which there was added a considerable percentage of Streptococcus, b, developed a pleasant flavor, an improvement upon the acid flavor of *Bacterium lactis acidi* alone, although it was not the Cheddar flavor. A third lot of experimental pasteurized-milk cheeses was made, in which the value of Streptococcus, b, in various proportions, together with *B. lactis acidi*, b, was tested. Three vats of cheese were made on each of three successive days. In series A, 50 per cent or more of the starter was a culture of Streptococcus, b; in series B this organism made up 33⅓ per cent or less of the starter. In series C the starter was a pure culture of *B. lactis acidi*, b. In Table XVI the scores for the cheeses are given, as determined by expert judges. The average score of the three judges for each cheese is given, 100 being perfect.

TABLE XVI.—*The effect upon flavor development of the use of various percentages of Streptococcus, b, in the starter for pasteurized-milk cheese*

| Cheese No. | Series A. | | Series B. | | Series C. | |
|---|---|---|---|---|---|---|
| | Inoculation. | Score. | Inoculation. | Score. | Inoculation. | Score. |
| 30 | Bacterium lactis acidi, b (50 per cent); Streptococcus, b (50 per cent). | 92.4 | Bacterium lactis acidi, b (66⅔ per cent); Streptococcus, b (33⅓ per cent). | 92.7 | Bacterium lactis acidi, b (100 per cent). | 90.9 |
| 31 | Bacterium lactis acidi, b (50 per cent); Streptococcus, b (50 per cent). | 90.2 | .....do..... | 93.5 | .....do..... | 91.7 |
| 32 | Bacterium lactis acidi, b (50 per cent); Streptococcus, b (50 per cent). | 94.0 | Bacterium lactis acidi, b (75 per cent); Streptococcus, b (25 per cent). | 93.8 | .....do..... | 91.0 |
| | Average..... | 92.2 | ..... | 93.3 | ..... | 91.2 |

It will be observed that for every day's make the cheese of series B, with the smaller percentages of Streptococcus, scored higher than series C, with the pure *Bacterium lactis acidi* starter. Series A, with the larger percentages of Streptococcus, scored higher than series C in two out of the three cheeses. Series B averaged 2.1 points higher than the series C, and series A averaged 1 point higher than series C.

The differences in flavor, however, were greater than the figures indicate, since the scores were made upon the commercial value of the cheese. The cheeses of series C had the acid flavor typical of a young pasteurized-milk cheese made with the ordinary commercial starter. The cheeses of series B had none of this acid flavor, but instead they possessed a mild flavor which was more agreeable to the taste of a number of persons knowing nothing of the experiment, to whose judgment the samples were submitted. All were agreed that the cheeses of series B lacked the acid taste. The cheeses of series A also differed greatly from those of the series C. They did not have the pleasant flavor of series B, however, but had an acid flavor essentially different from that of series C and inferior to the mild flavor of series B.

These experiments with the use of the Streptococcus, b, culture in starters demonstrate the fact that the addition of comparatively small percentages of this kind of culture brings about a decided difference in flavor, which is regarded as an improvement by all who have passed judgment in the matter. The larger percentages of streptococci give less desirable flavors than the smaller percentages. It seems probable that further experimentation with this and other cultures of Streptococcus, b, in combination with *Bacterium lactis acidi* may give results which will be of practical value in the improvement of flavors in pasteurized-milk cheese. And it does not seem unreasonable to hope that further experimentation with the *B. lactis acidi*, a or b, together with smaller percentages of Streptococcus, b, as the basis for a starter and with various combinations of very small percentage of the other cheese organisms, Cheddar flavors may be obtained in the pasteurized-milk cheese.

## SUMMARY

(1) The organisms constantly found in Cheddar cheese in such numbers as to indicate they must function in the ripening process are included in four groups: First, the *Bacterium lactis acidi*; second, the *B. casei*; third, Streptococcus; fourth, Micrococcus.

(2) On the basis of the fermentation powers each of the four groups may be divided into a number of varieties.

(3) The distribution of the varieties of the four groups in Cheddar cheese prepared from raw milk has been studied, as has also been done with cheese prepared from pasteurized milk.

(4) The flora of raw-milk cheese is varied and consists of all the varieties into which the four groups were divided.

(5) The flora of pasteurized-milk cheese, with the exception of the *Bacterium casei* group, is dependent upon the flora of the starter.

(6) The *Bacterium casei* group is apparently responsible for the pungent taste that develops late in the ripening period of both raw-milk and pasteurized-milk cheeses. It is probable that growth of this group continues during the major part of the ripening period.

(7) The action of two or more organisms growing together is not the sum of their individual actions when growing alone. When growing together, they may attack substances that neither can attack alone, or they may produce a larger quantity of acid than the sum of the quantities that either can produce alone.

(8) When added to pasteurized milk, the organisms of the *Bacterium casei* group produce a sour taste in the cheese during the early part of the ripening period.

(9) No Cheddar flavor is obtained in pasteurized-milk cheese when the organisms of the *Bacterium lactis acidi* group alone are used as starters. The varieties that are able to ferment the more complex substances are likely to produce a bitter taste.

(10) Starters composed of both *Bacterium lactis acidi*, b, and Streptococcus, b, when added to pasteurized milk, improve the quality of the cheese. It does not seem unreasonable to hope that starters may be obtained that will give the characteristic Cheddar flavor to the cheese prepared from pasteurized milk.

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# RELATION OF THE ACTION OF CERTAIN BACTERIA TO THE RIPENING OF CHEESE OF THE CHEDDAR TYPE [1]

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## INTRODUCTION

The ripening of Cheddar as well as other varieties of cheese has been studied by a large number of investigators. The decomposition of the protein and the nitrogenous substances thereby produced have been quite thoroughly studied in Europe and America. These studies have involved both hard and soft cheeses. The nature of the nonnitrogenous substances formed during fermentation in cheese, such as fatty acids, alcohol, esters, and aldehydes, has received less attention, but there can be no doubt that they contribute to the aroma and also to the taste of the product. In their relation to flavor they are equally, if not more, important than the nitrogenous substances.

According to present views, the factors involved in the curing of Cheddar cheese are the pepsin contained in the rennet; the activating lactic acid formed from lactose fermentation; galactase, the proteolytic enzym of milk; other inherent enzyms of milk; and certain biological agents other than those simply concerned in the first lactose fermentation.

Investigations at the Wisconsin Agricultural Experiment Station and the New York (Geneva) Agricultural Experiment Station have shown that the inherent enzyms of milk and rennet fail to produce the typical Cheddar cheese flavor. This has led to a more extensive investigation of the biological factors of Cheddar cheese ripening.

In an earlier publication from the Wisconsin station (Suzuki, Hastings, and Hart, 1910) [2] both volatile acids and esters were separated and identified from curing Cheddar cheese, but no data concerning the factors operative in their origin were presented. In a later publication (Hastings, Evans, and Hart, 1912) work was reported that showed the presence and persistence in this type of cheese of three groups of organisms, the *Bacterium lactis acidi* group, the *B. casei* group,[3] and possibly a group of coccus forms.

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[1] Work of the Department of Agriculture in cooperation with Wisconsin Agricultural Experiment Station.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 214–216.

[3] The organisms of the *Bacterium casei* group appear in the literature under a number of names, the most common being "lactic bacilli," "*Bacterium bulgaricus*" or "*Bacterium bulgaricum*," "*Bacterium casei*," and the "youghurt bacillus." The name "*Bacterium casei*" will be used in this article.

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In a preliminary investigation of the nonnitrogenous constituents of Cheddar cheese (unpublished data) the very pronounced differences that were expected in the quantity and variety of volatile acids, esters, and alcohols in good and poor types of cheese were not found. But since there were certain differences which could be only of biological origin, it was believed essential to this problem that the substances formed by the specific groups of organisms normally present in cheese be more carefully studied. For this reason it was decided to extend the investigation to an examination of the substances produced by representatives of the groups that had been found to be present in cheese in such numbers that it was evident that they must be of importance in the ripening process. In this way it was hoped to find the groups of organisms to which might be assigned responsibility for the production of definite nonnitrogenous compounds that could be correlated with flavor production. The compounds particularly sought were the alcohols and esters and caproic and butyric acids. Formic, acetic, propionic, lactic, and succinic acids were also included in the list of substances to be isolated. To some extent the sources of these bodies were also studied. This paper is a progress report on this phase of our work.

Ferdinand Cohn (1875) was the first to connect the cheese-ripening process with the activity of bacteria. Duclaux (1894, p. 265–267) considered that the volatile fatty acids found in cheese arose from the action of the bacteria on casein and from the hydrolysis of the fat. He believed also that butyric acid was a source of other volatile acids, the butyric acid arising partly from fat decomposition and partly from decomposition of casein. Baier (1895), Von Klecki (1896), and Weigmann (1896, 1898) believed butyric-acid bacteria to be of importance in the ripening of cheese. Von Freudenreich (1897, 1902) attributed to the lactic-acid bacteria the principal rôle in the ripening process, especially in Emmenthaler cheese. Jensen (1904) in his work on Emmenthaler and other European cheeses has contributed much to the general subject of the chemistry and bacteriology of cheese ripening and in agreement with Von Freudenreich gives to the lactic-acid-producing organisms very great importance in the ripening process. Suzuki, Hastings, and Hart (1910) have investigated the source of the volatile acids and the forms of lactic acid found in American Cheddar cheese, studying in connection with these subjects the decomposition of lactose, lactates, fat, proteins, and glycerin.

The constituents of a fresh cheese mass which can be sources of the nonnitrogenous bodies under consideration are paracasein, fat, lactose, lactates, and citrates. From paracasein there arises gradually during the ripening process a series of nitrogenous compounds which have been fairly well investigated (Winterstein; Steinegger; Benecke and Schulze; Van Slyke and Hart, Apr., 1903, and July, 1903; Dox). At least three of these—namely, cadaverin, putrescin, and ammonia—are slightly

volatile and probably can influence the aroma of cheese. The other nitrogenous end-products undoubtedly are factors in the flavor production, and influence taste.

It is known that proteolysis gives rise also to volatile fatty acids, particularly butyric acid. In addition, milk fat, which is present to a large extent in the cheese, is a source of caproic and butyric acids through bacterial and enzymic action. The glycerin of the fat after hydrolysis by biological agencies is a source of acetic and propionic acids under the influence of further fermentation (Suzuki, Hastings, and Hart, 1910). That decomposition of fat occurs during cheese ripening, giving rise to caproic and butyric acids, has been shown by a number of workers. Duclaux (1894, p. 286) found that this occurred to quite an extent, giving rise to free volatile fatty acids. Weigmann and Backe (1898) point to the presence in ripe cheese of free nonvolatile acids, such as oleic, palmitic, and stearic, as an indication of fat decomposition in the cheese-ripening process. Kirsten (1898, p. 1), however, thought these higher acids could arise from paracasein and claimed that fat decomposition in ripening cheese is almost imperceptible. Jensen (1904, p. 319) has shown that very probably fat decomposition does take place with production of fatty acids during cheese ripening. The lactose fermentation produces, besides lactic acid, formic and propionic acids, and under certain conditions butyric and caproic acids also are formed (Suzuki, Hastings, and Hart, 1910). Calcium lactate, according to Fitz (1878, p. 51; 1879, p. 479; 1880, p. 1309; 1881, p. 1084), is a source of acetic and propionic acids, and under certain conditions also of caproic and valeric acids. Jensen (Von Freudenreich and Jensen, 1906, p. 320) and Troili-Petersson (1909, p. 333) have shown that the lactates in Emmenthaler cheese are fermented by organisms with the production of propionic and acetic acids and $CO_2$. Troili-Petersson has also shown that glycerin may be a source of propionic acid.

In an extended investigation (Evans, Hastings, and Hart, 1914) of the flora of American Cheddar cheese it has been shown that the organisms fall into four groups, the *Bacterium lactis acidi*, the *B. casei*, and two coccus groups.

The substances produced by the coccus groups form the principal theme of this paper. In addition, data are given on the substances formed from two representatives of the *Bacterium casei* group. In the following work pure cultures of several of the coccus forms known to occur in American Cheddar cheese were inoculated into flasks containing 300 c. c. of sterile separated milk and kept at a temperature of 35° C. for at least two months before being examined. No alkali whatever was added to the milk. The high-acid-producing organisms (*B. casei* group) were also inoculated into flasks of milk similarly prepared and incubated. Each culture was put up in duplicate flasks. The methods of analysis used were those described by Suzuki, Hastings, and Hart (1910). All

flasks subjected to analysis were examined to ascertain their freedom from growth of other organisms.

It has been observed that active lactic acid is the main form of this acid in fresh cheese curd, but that it rapidly changes to the racemic variety. In addition to the foregoing studies on substances formed by bacteria, this paper also includes some work done on the agencies which cause these changes in the form of lactic acid present in cheese and which take place during the earlier period of cheese ripening.

In the preceding article (Evans, Hastings, and Hart) the presence of coccus forms in normal Cheddar cheese is demonstrated. It is shown that nonliquefying cocci which ferment lactose in milk cultures are always present, in percentage of the total bacterial content ranging upward to 50. The cocci are distinguished from the *Bacterium lactis acidi* group by their morphology and by the extent of reduction of litmus in milk cultures. In cultures of the *B. lactis acidi* group the cells are in pairs, and some or all of the cells are elongated; there is always a characteristic reduction of litmus. The cocci include those cultures in which the cells are spherical. The complete reduction of litmus beneath the surface layer, characteristic of the *B. lactis acidi* group, does not take place.

A classification of the cocci occurring in this type of cheese is made. They are divided into two groups on the basis of morphology: Streptococci and micrococci. Those occurring in pairs are included with the streptococci, together with those which form chains of varying lengths. The microccocci are the Coccaceæ which divide in two planes; consequently the cells appear in pairs, fours, or bunches. Most of the cultures of this group produce a heavy growth upon agar slant, which is often of some shade of yellow. A further differentiation of the groups into varieties is made on the basis of fermentation of the following test substances: Lactose, salicin, sucrose, glycerin, and mannit. This classification of the cocci is given in Table I. The substances produced by representatives of several of these varieties have been analyzed, and the data are presented in Tables II to X.

TABLE I.—*Differentiation of the coccus groups into varieties*

| Group. | Variety. | Production of acid in— | | | | |
|---|---|---|---|---|---|---|
| | | Lactose. | Salicin. | Sucrose. | Glycerin. | Mannit. |
| Streptococcus | a | − | − | − | − | − |
| | b | + | − | + | + | − |
| | c | + | + | + | + | − |
| | d | + | + | + | + | + |
| Micrococcus | b | + | + | − | − | − |
| | c | + | + | + | + | − |
| | d | + | + | − | − | + |

## ANALYSIS OF THE DECOMPOSITION PRODUCTS OF STERILE MILK

In Table II are given the quantities of the various substances found in 300 c. c. of the sterile milk after incubation for four months.

TABLE II.—*Decomposition products found in 300 c. c. of sterile milk*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity. | | |
|---|---|---|---|
| | Flask 1. | Flask 2. | Average. |
| Total volatile acids | 11. 763 | 12. 567 | 12. 164 |
| Formic acid | 5. 748 | 5. 348 | 5. 548 |
| Acetic acid | 5. 566 | 6. 756 | 6. 160 |
| Propionic acid | . 000 | . 000 | . 000 |
| Butyric acid | . 000 | . 000 | . 000 |
| Caproic acid | . 449 | . 463 | . 456 |
| Acids from alcohols | . 954 | . 650 | . 802 |
| Acids from esters | . 685 | . 600 | . 640 |
| Succinic acid | . 000 | . 000 | . 000 |
| Total lactic acid | . 000 | . 000 | . 000 |
| Racemic lactic acid | | | |
| Active lactic acid | | | |

The occurrence of formic and acetic acid in the controls may be due to the decomposition of lactose in the process of sterilization. Formic acid, at least, has been observed in milk heated for some time at high temperature (Cazeneuve and Haddon).

The cultures of Streptococcus $b_1$ (Table III) were 8 weeks old when analyzed. Little digestion of the medium was apparent. The medium had a clean, sweet, fruity, or nutty taste and odor, was gray white in color, and somewhat slimy. This organism was present in the cheese to the extent of 10,000,000,000 per gram when isolated. The cheese was 77 days old when examined. It had a very mild Cheddar flavor, which developed late in the curing, and it afterwards developed a good sharp flavor.

TABLE III.—*Substances formed by the action of Streptococcus $b_1$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 52. 689 | 54. 930 | 12. 164 | 40. 525 | 42. 766 |
| Formic acid | . 000 | . 000 | 5. 548 | −5. 548 | −5. 548 |
| Acetic acid | 49. 920 | 50. 810 | 6. 160 | 43. 760 | 44. 650 |
| Propionic acid | 2. 575 | 4. 120 | . 000 | 2. 575 | 4. 120 |
| Butyric acid | . 000 | . 000 | . 000 | | |
| Caproic acid | . 194 | . 000 | . 456 | | |
| Acids from alcohols | . 769 | . 621 | . 802 | | |
| Acids from esters | . 850 | . 840 | . 640 | . 210 | . 200 |
| Succinic acid | . 000 | . 000 | . 000 | | |
| Total lactic acid | Trace. | . 000 | . 000 | | |
| Racemic lactic acid | | | | | |
| Active lactic acid | | | | | |

Streptococcus $b_1$ decomposed all the formic acid present in the milk and produced large quantities of acetic and a little propionic acid. Esters were produced in small amounts. No lactic acid was found.

The cultures of Streptococcus $b_2$ (Table IV) were 11 weeks old when analyzed. In both flasks a soft curd was deposited. The contents of flask 1 had a sharp nutty odor and flavor. Flask 2 had a sharp, acid, unpleasant taste and a sharp, rancid smell suggesting butyric acid. The cheese from which the isolation was made was 101 days old when examined. It contained this organism in numbers of 1,000,000,000 per gram. The cheese possessed a good Cheddar flavor when 2 weeks old. Later, a sharpness developed, but the cheese remained good for 6 months.

TABLE IV.—*Substances formed by the action of Streptococcus $b_2$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 49.138 | 55.152 | 12.165 | 36.973 | 42.987 |
| Formic acid | 3.640 | 6.333 | 5.548 | −1.908 | .789 |
| Acetic acid | 38.370 | 41.059 | 6.160 | 32.210 | 34.899 |
| Propionic acid | 4.903 | 5.138 | .000 | 4.903 | 5.138 |
| Butyric acid | .558 | .563 | .000 | .558 | .563 |
| Caproic acid | 1.667 | 2.059 | .456 | 1.211 | 1.603 |
| Acids from alcohols | 8.607 | 3.552 | .802 | 7.805 | 2.750 |
| Acetic acid | 8.209 | 3.187 | ..... | 7.407 | 2.385 |
| Propionic acid | .398 | .365 | ..... | .398 | .365 |
| Acids from esters | .250 | 1.930 | .640 | ..... | 1.290 |
| Total lactic acid | .000 | .000 | .000 | .000 | .000 |
| Racemic lactic acid | ..... | ..... | ..... | ..... | ..... |
| Active lactic acid | ..... | ..... | ..... | ..... | ..... |

This form of coccus decomposed a part of the formic acid originally present in the medium. The increase in acidity was mainly due to acetic acid, but some propionic and a little caproic acid were also formed. The interesting point in connection with this organism, however, was the strong production of alcohols, amounting to a quantity equivalent to nearly 8 cubic centimeters of decinormal acid. Most of this alcohol was ethyl, a little propyl alcohol making up the remainder. In one flask a marked production of esters was also noted. No lactic acid was produced.

The cultures of Streptococcus $b_3$ (Table V) were 4½ months old when analyzed. Flask 1 had a yellowish colored solution over a firmly deposited custard-like curd. The solution was acid to litmus and had a pleasant, slightly acid smell. The residue in flask 2 was less than that in flask 1 and was covered by a brown-colored solution which was acid to litmus. Its odor was similar to that of flask 1, but was more pronounced, giving a suggestion of cheese odor.

TABLE V.—*Substances formed by the action of Streptococcus $b_3$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 88.799 | 90.474 | 14.530 | 74.269 | 75.944 |
| Formic acid | 3.153 | .000 | 8.295 | −5.142 | − 8.295 |
| Acetic acid | 68.190 | 69.150 | 6.135 | 62.055 | 63.015 |
| Propionic acid | 10.209 | 9.970 | .000 | 10.209 | 9.970 |
| Butyric acid | 2.090 | 3.821 | .000 | 2.090 | 3.821 |
| Caproic acid | 5.157 | 7.533 | .100 | 5.057 | 7.433 |
| Acids from alcohol | 7.743 | 5.158 | .375 | 7.368 | 4.783 |
| Formic acid | .313 | .000 | ..... | .313 | ..... |
| Acetic acid | 6.908 | 4.612 | ..... | 6.533 | ..... |
| Propionic acid | .522 | .546 | ..... | .522 | .546 |
| Acids from esters | 4.739 | 4.856 | .833 | 3.906 | 4.023 |
| Formic acid | .443 | .000 | ..... | .443 | .000 |
| Acetic acid | 3.979 | 4.651 | ..... | 3.146 | 3.818 |
| Propionic acid | .317 | .205 | ..... | .317 | .205 |
| Citric acid | 65.982 | 47.090 | 84.800 | −18.818 | −37.710 |
| Total lactic acid | .000 | .000 | .000 | .000 | .000 |
| Racemic lactic acid | ..... | ..... | ..... | ..... | ..... |
| Active lactic acid | ..... | ..... | ..... | ..... | ..... |
| Ammonia .....grams | .045 | .052 | .023 | .022 | .029 |

The data in Table V show the same general indications as the data in Table IV. A larger increase was shown in total volatile acidity. Comparatively a much greater increase was noted in the case of butyric and caproic acids. A greater increase was also evident in alcohol and ester production, ethyl alcohol and acetic acid in ester combination predominating. A minute quantity of formic acid previously existing in ester compounds and also from methyl alcohol was recovered. Since probably only about 6.2 per cent of esters are recovered in the method used, the amount of esters actually found indicates a preexisting quantity of those bodies equivalent to 64.8 c. c. N/10. This quantity is greater than the ester content of any cheese examined. If this organism is an agent which produced esters in cheese, as the data indicate, it would, however, be subjected to inhibiting influences in the cheese mass and probably not be able to form esters in such great quantities as when in pure culture.

The culture of Streptococcus $d_1$ (Table VI) was 2½ months old when analyzed. The medium had a pleasant nutty odor and a slightly acid taste. A soft custard-like curd had formed; that in flask 2 showed the greater bacterial action, having a more acid odor, but the taste was similar to that in flask 1. The curds were alike, with a clear supernatant liquid. The cheese from which this organism was isolated was 133 days old and contained the organism to the extent of 170,000,000 per gram. It had a mild Cheddar flavor after 4 months of curing.

TABLE VI.—*Substances formed by the action of Streptococcus $d_1$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 19.142 | Lost. | 14.530 | 4.612 | Lost. |
| Formic acid | .000 | ..... | 8.295 | −8.295 | ..... |
| Acetic acid | 18.310 | ..... | 6.135 | 12.175 | ..... |
| Propionic acid | .692 | ..... | .000 | .692 | ..... |
| Butyric acid | .000 | ..... | .000 | ..... | ..... |
| Caproic acid | .140 | ..... | .100 | ..... | ..... |
| Acids from alcohol | .680 | 1.000 | .375 | ..... | 0.625 |
| Acids from esters | .915 | .725 | .833 | .082 | ..... |
| Citric acid | ..... | ..... | 84.800 | ..... | ..... |
| Total lactic acid | ..... | Trace. | .000 | ..... | ..... |
| Racemic lactic acid | ..... | ..... | ..... | ..... | ..... |
| Active lactic acid | ..... | ..... | ..... | ..... | ..... |

All the formic acid was destroyed by this coccus, and but a comparatively small quantity of acetic acid formed. The activity of this organism was apparently slight; but slight traces of esters were found in one flask and no lactic acid in either flask. The apparent contradiction that there exists a larger content of acetic acid than total volatile acids is due to the destruction of formic acid.

The cultures of Streptococcus $d_2$ (Table VII) were 2 months old when analyzed. They possessed a pleasant nutty taste and smell. No digestion was apparent. The contents of flask 2 had a trifle more pronounced flavor and odor than in flask 1, but were of the same quality. The cheese from which the organism was isolated was 75 days old and contained this coccus to the extent of 10,000,000,000 per gram. No typical Cheddar flavor had developed. After 5 months the cheese developed a sharpness in taste, but still was fairly good.

TABLE VII.—*Substances formed by the action of Streptococcus $d_2$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 58.313 | 66.664 | 14.530 | 43.783 | 52.134 |
| Formic acid | .789 | 1.029 | 8.295 | −7.506 | −7.266 |
| Acetic acid | 53.140 | 59.530 | 6.135 | 47.005 | 53.395 |
| Propionic acid | 4.029 | 5.700 | .000 | 4.020 | 5.700 |
| Butyric acid | .000 | .301 | .000 | ..... | ..... |
| Caproic acid | .355 | .104 | .100 | ..... | ..... |
| Acid from alcohols | .800 | .765 | .375 | .425 | .390 |
| Acid from esters | .770 | .540 | .833 | ..... | ..... |
| Citric acid | 7.100 | 7.100 | 84.800 | −77.700 | −77.700 |
| Total lactic acid | .000 | .000 | .000 | ..... | ..... |
| Racemic lactic acid | ..... | ..... | ..... | ..... | ..... |
| Active lactic acid | ..... | ..... | ..... | ..... | ..... |

This culture produced acetic acid almost entirely. As practically all of the citric acid had been destroyed, it may be assumed that this acid was in part the source of the acetic acid. That citric acid can be broken down by certain organisms has already been pointed out by Bosworth and Prucha (1910).

The cultures of Micrococcus b (Table VIII) were 3¼ months old when analyzed. The flasks were alike in appearance and odor. The cheese was 43 days old and had a mild Cheddar flavor when the isolation was made. This organism was present in the cheese to the extent of 1,600,000 per gram.

TABLE VIII.—*Substances formed by the action of Micrococcus b*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 72.785 | 67.529 | 12.164 | 60.621 | 55.365 |
| Formic acid | 4.857 | 3.667 | 5.548 | −.621 | −1.881 |
| Acetic acid | 64.305 | 59.740 | 6.160 | 58.145 | 53.580 |
| Propionic acid | 3.220 | 3.914 | .000 | 3.220 | 3.914 |
| Butyric acid | .000 | .000 | .000 | .000 | .000 |
| Caproic acid | .403 | .208 | .456 | ..... | ..... |
| Acids from alcohol | .409 | Lost. | .802 | ..... | ..... |
| Acids from esters | .500 | .405 | .640 | ..... | ..... |
| Succinic acid | .000 | .000 | .000 | .000 | .000 |
| Total lactic acid | 17.152 | .000 | .000 | 17.152 | .000 |
| Racemic lactic acid | 3.730 | .000 | .000 | 3.730 | .000 |
| Active lactic acid | 13.422 | .000 | .000 | 13.422 | .000 |

Acetic acid shows the only large increase among the volatile acids. In flask 1 a small quantity of lactic acid had developed. Most of it was of the active variety.

The cultures of Micrococcus d (Table IX) were 1¾ months old when analyzed. Flask 2 showed from its appearance and odor further decomposition and probably more rapid growth of the organism than flask 1.

TABLE IX.—*Substances formed by the action of Micrococcus d*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 29.482 | 26.614 | 12.164 | 17.318 | 14.450 |
| Formic acid | 2.484 | 1.316 | 5.548 | −3.064 | −4.232 |
| Acetic acid | 8.370 | 13.295 | 6.160 | 2.210 | 7.135 |
| Propionic acid | 4.540 | 4.988 | .000 | 4.540 | 4.988 |
| Butyric acid | 7.811 | 2.456 | .000 | 7.811 | 2.456 |
| Caproic acid | 6.277 | 4.559 | .456 | 5.821 | 4.103 |
| Acids from alcohol | 1.499 | 1.752 | .802 | .697 | .950 |
| Acids from esters | .350 | .450 | .640 | ..... | ..... |
| Succinic acid | .830 | 3.300 | .000 | .830 | 3.300 |
| Total lactic acid | 3.396 | 20.856 | .000 | 3.396 | 20.856 |
| Racemic lactic acid | .000 | 11.940 | .000 | .000 | 11.940 |
| Active lactic acid | 3.396 | 8.916 | .000 | 3.396 | 8.916 |

It will be noticed that formic acid has decreased. This will be found true for all the organisms studied, the acid probably being decomposed by the organisms themselves. In flask 1 the greatest increase is shown in the butyric-acid content. In flask 2, where greater decomposition and probably more rapid growth of the organisms occurred, the butyric acid is very much less, while the acetic acid has increased. This would indicate a decomposition of butyric acid to a lower acid, as Duclaux suggests in his theory of the formation of acids lower than butyric. All of the volatile acids, except formic, show an increase. A very small quantity of succinic acid was formed, but no esters and very little alcohol were produced. In flask 2 there was quite an amount of lactic acid, of which most was racemic.

The substances formed by a third Micrococcus are given in Table X. This culture was not classified as to variety. It was isolated from a cheese when the latter was 44 days old and when the organism was present in numbers amounting to 100,000,000 per gram. After 5 months this cheese developed a good Cheddar flavor. In both flasks the media were light brown in color and had a pleasant nutty odor and taste; they showed slight digestion and were but slightly acid to litmus.

TABLE X.—*Substances formed by the action of an unidentified Micrococcus*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 46.058 | 52.669 | 14.530 | 31.538 | 38.139 |
| Formic acid | .000 | .000 | 8.295 | −8.295 | −8.295 |
| Acetic acid | 41.850 | 37.060 | 6.135 | 35.715 | 30.925 |
| Propionic acid | 3.999 | 14.886 | .000 | 3.999 | 14.886 |
| Butyric acid | .138 | .289 | .000 | .138 | .289 |
| Caproic acid | .081 | .434 | .100 | ..... | ..... |
| Acids from alcohols | .990 | .700 | .375 | .525 | .325 |
| Acids from esters | 1.000 | .540 | .833 | .167 | ..... |
| Citric acid | 80.900 | 59.240 | 84.800 | ..... | −25.560 |
| Total lactic acid | .000 | .000 | .000 | .000 | .000 |
| Racemic lactic acid | ..... | ..... | ..... | ..... | ..... |
| Active lactic acid | ..... | ..... | ..... | ..... | ..... |

This organism produced quite a quantity of acetic acid and more propionic acid than any other organism examined. No lactic acid was found.

In order to determine the influence of the presence of alkali on the character of the products formed, a flask of milk to which was added calcium carbonate was inoculated with one of the micrococci. The substances formed were acetic, propionic, butyric, and caproic acids, but no formic acid. The proportion of these acids was very similar to that of the acid formed by Micrococcus d and would indicate that the alkali exerted no influence on the character of the substances formed.

From a summary of all the foregoing data it appears that the coccus forms do not produce formic acid, and, with the exception of Micrococcus b and d, do not produce lactic acid. In the case of these two strains the form of acid produced was both active and racemic. With the exception of Micrococcus d all produce relatively large amounts of acetic acid. Streptococcus $b_3$ produced a fairly large quantity of butyric and caproic acids.

## SUBSTANCES FORMED BY ORGANISMS OF THE BACTERIUM CASEI GROUP

In Tables XI and XII are given data showing the substances formed by the action of a high-acid-producing organism, one of the *Bacterium casei* group. Duplicate flasks $58_1$ and $58_2$ were prepared from two strains of the same culture obtained from different colonies on an agar plate. The milk media at 7½ months old were light yellow in color, and the curd had settled in a firm mass. Both flasks of $58_1$ and flask 1 of $58_2$ had a very faint acid odor. Flask 2 of $58_2$ had a ripened-cream odor.

TABLE XI.—*Substances formed by the action of culture $58_1$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 35.128 | 38.063 | 14.530 | 20.598 | 23.533 |
| Formic acid | .000 | 1.387 | 6.295 | −6.295 | −4.908 |
| Acetic acid | 33.420 | 35.760 | 6.138 | 27.285 | 29.625 |
| Propionic acid | 1.708 | .916 | .000 | 1.708 | 0.916 |
| Butyric acid | .000 | .000 | .000 | ..... | ..... |
| Caproic acid | .000 | .000 | .100 | ..... | ..... |
| Acids from alcohol | .250 | Lost. | .375 | ..... | ..... |
| Acids from esters | .800 | Lost. | .833 | ..... | ..... |
| Citric acid | .000 | .000 | 84.800 | −84.800 | −84.800 |
| Total lactic acid | 92.648 | 99.100 | .000 | 92.648 | 99.100 |
| Racemic lactic acid | 44.552 | 87.800 | ..... | 44.552 | 87.800 |
| Active lactic acid | 48.096 | 11.300 | ..... | 48.096 | 11.300 |

TABLE XII.—*Substances formed by the action of culture $58_2$*

[Computed in cubic centimeters of tenth-normal solution]

| Substance. | Quantity found. | | Control. | Quantity produced. | |
|---|---|---|---|---|---|
| | Flask 1. | Flask 2. | | Flask 1. | Flask 2. |
| Total volatile acids | 41.675 | 40.928 | 14.530 | 27.145 | 26.498 |
| Formic acid | 3.408 | .984 | 8.295 | −4.887 | −7.311 |
| Acetic acid | 36.130 | 31.990 | 6.135 | 29.995 | 25.855 |
| Propionic acid | 2.137 | 7.954 | .000 | 2.137 | 7.954 |
| Butyric acid | .000 | .000 | .000 | ..... | ..... |
| Caproic acid | .000 | .000 | .100 | ..... | ..... |
| Acids from alcohol | .500 | .950 | .375 | ..... | ..... |
| Acids from esters | 1.500 | 1.000 | .833 | .667 | .167 |
| Citric acid | .000 | .000 | 84.800 | −84.800 | −84.800 |
| Total lactic acid | 48.540 | 39.970 | .000 | 38.540 | 39.970 |
| Racemic lactic acid | 41.100 | 20.170 | ..... | 41.100 | 20.170 |
| Active lactic acid | 7.440 | 19.800 | ..... | 7.440 | 19.800 |

This organism, as might be expected, shows a marked difference from the coccus group in the character of the substances formed. A large amount of lactic acid, including both the racemic and the active forms, was produced. All the citric acid of the milk was destroyed. Like the coccus forms, this organism also produced much acetic acid, but no formic, butyric, or caproic acid. Culture $58_2$ produced some esters.

## ESTER FORMATION IN CHEDDAR CHEESE

It has been determined that esters do not appear in Cheddar cheese until it is about 5 weeks old. Streptococcus $b_3$ (see Table V) produced an ester content in the medium equivalent to 64.8 c. c. N/10. To throw some light on the question whether esters could be formed in the cheese or medium from mere mass action of free alcohol and acid, a trial was made with a mixture of these two substances. It is known that the contact of acetic acid and ethyl alcohol can produce esters even without adding a dehydrating agent. Dilute solutions of pure acid and pure alcohol were mixed and allowed to stand for a few months, and then a very slight excess of KOH solution was added. The alcohol and esters were next distilled off. The distillate was saponified with KOH, acidified with $H_2SO_4$, and distilled repeatedly to obtain the acids which had entered into the ester combination. Blank determinations were carried out to check the purity of all chemicals used. The results are given in Table XIII.

TABLE XIII.—*Production of ester from the contact of acid and alcohol*

| Ethyl alcohol. | Acetic acid. | Result. |
|---|---|---|
| *Per cent.* | *Per cent.* | |
| 1 | 1 | No ester detected. |
| 2 | 2 | Small amount of ester. |
| 5 | 5 | Esters formed. |
| 8 | 8 | Do. |
| 14 | 14 | Do. |

Table XIII shows that free acetic acid and alcohol can not form esters in dilute aqueous solutions. Comparing this concentration with that found in cheese, it is probable that the solution of alcohol in the cheese moisture is very dilute—much less than 1 per cent. The greater part of the acids is also combined with basic substances. If these assumptions are accepted, then it can be said that the esters in cheese are probably not produced by mere contact of alcohol and acid but by the intervention of biological activities.

Of course, the question of actual concentration of alcohol or acid in any phase in the cheese mass is not possible of definite statement. There may be very little "free" water in the cheese, most of it being in com-

bination with the cheese colloids; consequently the concentration of acid or alcohol in such water may be very large, thereby affording an opportunity for ester formation by mass action. On the other hand, it must not be assumed that the alcohols or acids are "free" in such a complex system, but may also be in combination with the colloids of the cheese mass. If this last alternative is permissible—and the writers believe it to be true, especially for the acids—then there is some reason, at least, for the assumption that ester formation is not the result of mere contact of acid and alcohol, but occurs through the intervention of some agent which shifts the point of equilibrium in the system toward ester stability.

To determine whether inherent milk enzyms acting in curing cheese could produce esters, alcohols, or volatile acids, a cheese was made from chloroformed milk and kept in an atmosphere of chloroform for 5 months. To determine the volatile bodies, 800 grams of the cheese were submitted to steam distillation, after acidifying with $H_2SO_4$. The entire analytical process was conducted as has been described. The results were negative, there being neither acids nor esters. This shows that inherent milk enzyms are not the cause of the production of volatile fatty acids and esters in curing cheese. From this experiment it is apparent that the inherent lipase in milk is either retarded in its action by chloroform or else is very slow in its action.

## AMMONIA PRODUCTION IN MILK

The origin of ammonia in ripening cheese had been ascribed by Babcock and Russell (1897, p. 161) and Babcock, Russell, Vivian, and Hastings (1899, p. 157) to the action of galactase. In further work on this problem Van Slyke and Hart (1903) showed that in chloroformed cheese, where galactase and pepsin would be the only proteolytic agents present, no ammonia was formed. To throw further light on this problem, cultures of a few of the organisms known to be active in Cheddar cheese were examined for ammonia production. Milk was the medium used for these determinations. A part of this medium, in the case of the coccus group, was distilled directly with MgO. In the case of *Bacterium casei*, the medium was first treated with tannic acid and salt solution according to the standard methods for separation of the tannin precipitate, and the ammonia determined in the filtrate by distillation with MgO. In Table XIV are recorded the results.

TABLE XIV.—*Quantity of ammonia produced in 300 c. c. of milk by different organisms*

| Organism. | Quantity of $NH_3$ found. | Quantity of $NH_3$ in control. | Quantity of $NH_3$ formed by organisms. |
|---|---|---|---|
| | *Grams.* | *Grams.* | *Grams.* |
| Streptococcus $b_2$ | 0.0220 | 0.023 | ..... |
| | .0200 | .023 | ..... |
| Streptococcus $b_3$ | .0450 | .023 | 0.0220 |
| | .0520 | .023 | .0290 |
| Micrococcus | .0690 | .023 | .0460 |
| Bacterium $casei_1$ | .0427 | .026 | .0167 |
| | .0382 | .026 | .0122 |
| Bacterium $casei_2$ | .0367 | .026 | .0107 |
| | .0453 | .026 | .0193 |

No large quantity of ammonia was formed by any of the organisms examined. The difference in the amount of ammonia produced by Streptococcus $b_2$ and Streptococcus $b_3$—two strains of the same variety—may be due to the fact that Streptococcus $b_3$ grew for more than twice as long a time as did the other. It is clear from Table XIV that some of the biological agencies active in the cheese are capable of forming both acids and ammonia.

## KINDS OF LACTIC ACID IN CHEESE[1]

In considering lactic acid and its changes in cheese, it will be remembered that lactose disappears from the cheese mass after a very few days of curing; subsequently the lactic acid increases up to the five weeks' stage. At later periods the lactic-acid content fluctuates, probably the result of production and decomposition by active organisms. Thus, there seems to be a source of lactic acid other than lactose. A solution of alanin, one of the amino acids arising from casein proteolysis and very closely related to lactic acid, was inoculated with a piece of old cheese, in order to ascertain whether alanin could be a source of lactic acid (Suzuki, Hastings, and Hart, 1910). The results were negative, but it is possible that either the nature of the solution or the age of the cheese was responsible for this result. Additional work on this point is necessary.

It is known that cheese contains lactic acid, which usually is racemic in variety. It has been shown in the preceding article that Cheddar cheese 4 or 5 days old contains both racemic and active lactic acid, the latter being present in much greater amount than the former. The active form gradually decreases until it disappears, while the racemic acid increases and remains. It was found by Salkowski (1909, p. 237) that the transformation of dextro lactic acid into racemic acid on pro-

[1] The work reported in the remainder of this paper was completed before the classification of cheese organisms referred to on page 195 and treated in detail in the preceding article entitled "Bacteria concerned in the production of the characteristic flavor of cheese of the Cheddar type" was adopted; consequently that classification is ignored in the following pages.

longed standing takes place in a meat extract such as Liebig's. In the curing of cheese the disappearance of active lactic acid, as well as the production of racemic lactic acid, takes place rapidly. The early stages of these phenomena were next investigated.

Whey drawn from the vat during the process of cheese making and subjected to analysis for lactic acid gave the results shown in Table XV:

TABLE XV.—*Analysis of whey, showing quantity of lactic acid as zinc lactate*

| Fraction No. | Crystals of zinc lactate. | Water of crystallization.[1] |
|---|---|---|
| | *Grams.* | *Per cent.* |
| 1 | 2.4587 | 13.07 |
| 2 | .7902 | 12.27 |

[1] The theoretical percentage for water of crystallization in active zinc lactate is 12.89.

Fresh curd from which the above whey was drawn was kept at 35° C. for 3 days and gave the following results (Table XVI):

TABLE XVI.—*Analysis of fresh curd, 3 days old, showing quantity of lactic acid as zinc lactate*

| Fraction No. | Crystals of zinc lactate. | Water of crystallization.[1] |
|---|---|---|
| | *Grams.* | *Per cent.* |
| 1 | 2.0837 | 17.99 |
| 2 | .1652 | 17.43 |

[1] The theoretical percentage for water of crystallization in racemic zinc lactate is 18.18.

It is seen that whey contained active lactic acid, while curd or cheese only 3 days old and kept at 35° C. contained nearly all its lactic acid in the racemic form. It is probable that a second group of organisms follows the early action of the predominating active lactic-acid producers in the cheese during the first 3 days. There is also a possibility that a somewhat different sequence of bacterial life occurs in the whey from that which takes place in the curd, with the result that active acid is produced in whey and the racemic variety in the curd. To settle this point, whey and curd were investigated for the forms of lactic acid occurring in them. The results are given in Table XVII:

TABLE XVII.—*Analysis of whey and curd, showing the quantity of lactic acid as zinc lactate*

| Fraction No. | Whey when drawn. | | Curd at hooping time. | |
|---|---|---|---|---|
| | Crystals of zinc lactate. | Water of crystallization. | Crystals of zinc lactate. | Water of crystallization. |
| | *Grams.* | *Per cent.* | *Grams.* | *Per cent.* |
| 1 | 0.4570 | 13.65 | 0.1974 | 12.36 |
| 2 | 1.6520 | 12.50 | ..... | ..... |

Table XVII shows that the whey and curd contained active lactic acid and had a similar course of fermentation during the very early stages. A different fermentation evidently took place in the cheese after pressing, but not in the curd stage. The next question that arose was, At what stage in the curing process did the production of racemic lactic acid take place? The data in Table XVIII show that racemic acid begins to appear very soon after going to press.

TABLE XVIII.—*Analysis of whey, curd, and cheese, showing the quantity of lactic acid as zinc lactate*

| Fraction No. | Whey when drawn. | | Curd at hooping time. | | Whey kept at temperature of curd and stood overnight in pressing room. | | Cheese 24 hours old. | | Cheese 48 hours old. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Crystals of zinc lactate. | Water of crystallization. | Crystals of zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. | Crystals of zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. |
| | *Grams.* | *Per ct.* | *Grams.* | *Per ct.* | *Grams.* | *Per ct.* | *Grams.* | *Per ct.* | *Grams.* | *Per ct.* |
| 1 | 0.2360 | 13.64 | 0.3044 | 13.00 | 0.6435 | 13.05 | 0.0533 | 17.07 | 0.1123 | 17.45 |
| 2 | .0000 | ..... | .0000 | ..... | .8010 | 13.54 | .0846 | 17.37 | .1420 | 17.11 |
| 3 | ..... | ..... | ..... | ..... | .2745 | 13.47 | .1260 | 17.77 | .5805 | 17.07 |
| 4 | ..... | ..... | ..... | ..... | .3863 | 12.96 | .2606 | 14.19 | .0565 | 14.69 |
| 5 | ..... | ..... | ..... | ..... | .2788 | 13.12 | .4948 | 13.29 | .4555 | 13.63 |
| 6 | ..... | ..... | ..... | ..... | .0975 | 13.53 | ..... | ..... | ..... | ..... |

Whey when drawn, and also after standing overnight, contained active lactic acid. Curds at hooping time contained active lactic acid. One-day-old and two-day-old cheese contains a mixture of racemic and active acid.

The causes for the early production of racemic acid and the disappearance of active acid may be ascribed to a direct production by either enzyms or bacterial action of active acid which is of opposite polarity from that already present.

In order to study the relation of enzymic action in curd to this problem, the following experiment was performed: Curd at hooping time was divided into five parts. The first portion was immediately analyzed for lactic acid; the second portion was analyzed after standing 46 hours in the pressing room; the third portion was kept for 17 days at 35° C.; the fourth part was treated with chloroform and kept for 17 days at room temperature; the fifth portion was treated with chloroform and stood for 3 months at room temperature. The data secured on the nature of the lactic acid produced are shown in Table XIX.

TABLE XIX.—*Analysis of curd, showing lactic acid as zinc lactate*

| Fraction No. | Curd at hooping time. | | Curd kept 46 hours in pressing room. | | | Curd kept 46 hours at 35° C. | | Curd kept 17 days with chloroform. | | Curd kept 3 months with chloroform. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. | Zinc oxid in lactate. | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. |
| | *Grams.* | *P. ct.* | *Grams.* | *P. ct.* | *P. ct.* | *Grams.* | *P. ct.* | *Grams.* | *P. ct.* | *Grams.* | *P. ct.* |
| 1 | 0.1902 | 12.92 | 0.5601 | 15.97 | 33.8 | 0.6515 | 18.00 | 0.2637 | 12.96 | 0.1490 | 17.92 |
| 2 | .0000 | ..... | .5854 | 14.14 | 33.9 | .6040 | 18.11 | .0000 | ..... | .2183 | 14.13 |
| 3 | ..... | ..... | .2314 | 13.48 | ..... | .3901 | 17.86 | ..... | ..... | .2119 | 12.79 |
| 4 | ..... | ..... | .0000 | ..... | ..... | .0000 | ..... | ..... | ..... | 1.0895 | 12.78 |
| 5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .7685 | ..... |
| 6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .2074 | 13.21 |
| 7 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .1546 | 13.06 |

In order to verify the results secured on the increase of racemic acid and the decrease of active acid in fresh cheese curd, as shown above, another sample of fresh curd was divided into three portions. One portion was examined immediately for lactic acid, another after 24 hours, and the third portion after keeping at 60° for 48 hours. See Table XX.

TABLE XX.—*Analysis of curd and fresh cheese, showing lactic acid as zinc lactate*

| Fraction No. | Fresh curd. | | | One-day-old cheese. | | | Two-day-old cheese. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zinc lactate. | Water of crystallization. | Zinc oxide. | Zinc lactate. | Water of crystallization. | Zinc oxid. | Zinc lactate. | Water of crystallization. | Zinc oxid. |
| | *Grams.* | *Per cent.* | *Per cent.* | *Grams.* | *Per cent.* | *Per cent.* | *Grams.* | *Per cent.* | *Per cent.* |
| 1 | 0.2704 | 12.52 | 33.6 | 0.1786 | 17.19 | 32.7 | 0.1670 | 17.25 | 33.0 |
| 2 | .0617 | 11.80 | 33.4 | .0975 | 12.20 | 33.3 | .0457 | 17.07 | 33.2 |
| 3 | .0000 | ..... | ..... | .0778 | 12.90 | 33.9 | .1946 | 12.90 | 33.8 |
| 4 | ..... | ..... | ..... | .1514 | 12.30 | 33.8 | .2472 | 13.50 | 33.9 |
| 5 | ..... | ..... | ..... | .2133 | 12.00 | 33.6 | .0551 | 12.00 | 34.0 |
| Total. | .3321 | ..... | ..... | .7186 | ..... | ..... | .7096 | ..... | ..... |

The theoretical percentage for water of crystallization in racemic zinc lactate is 18.18; in active zinc lactate it is 12.89 per cent. The theoretical percentage of ZnO in anhydrous zinc lactate is 33.3.

From Table XX it is clear that in fresh curd, which contains active lactic acid, the production of racemic acid begins after about 24 hours at room temperature and that this production is accelerated by a temperature of 35° C. In the curd kept with chloroform for 17 days the production of racemic acid appeared to have been checked, while curds kept with chloroform for 3 months gave a small amount of the racemic variety. A parallel case was found by Saiki (1909) in the autolysis of a normal dog's liver, even in a strictly sterile solution. In his experiments racemic lactic acid gradually formed. This enzymic action may be considered a partial cause of the appearance of the racemic acid. The question whether enzymic action decomposes the lactose of the curd

into racemic acid or causes a production of an active acid of opposite polarity to the acid already present has not been settled. It has been shown (Hastings, Evans, and Hart) that the *Bacterium lactis acidi* does produce enzyms, and it may be that these enzyms are one of the factors in the production of racemic acid, although it is more probable that, because of the very slowness of the enzymic action, the real factor is an increasing number of active bacteria of different types from the *B. lactis acidi*.

The question whether *B. lactis acidi* or its enzym is the cause of the disappearance of active lactic acid and the appearance of racemic acid must be considered. It is known that lactic acid isolated from a lactose solution inoculated with *B. lactis acidi* is active in variety and not racemic. Even after prolonged standing, the lactic acid is found to be active. For this reason it is not believed that *B. lactis acidi* is the direct cause of this change. To determine whether the enzym of *B. lactis acidi* is the cause of this transformation, a solution containing active lactic acid, formed by inoculation with this organism and after several days treated with toluol, was allowed to stand for 2 months at 35° C. At the end of this time all of the lactic acid isolated was found to be active in variety, as shown by Table XXI.

TABLE XXI.—*Analysis of a solution of toluolated active lactic acid, showing active lactic acid*

| Fraction No. | Zinc lactate. | Water of crystallization. |
|---|---|---|
| | *Grams.* | *Per cent.* |
| 1..... | 0.1148 | 12.98 |
| 2..... | .4812 | 12.96 |
| 3..... | 1.0186 | 12.99 |
| 4..... | 1.3713 | 13.06 |

Further, it was thought possible that the kind of lactic acid produced by *B. lactis acidi* might be influenced by temperature conditions. In order to test this, a lactose solution containing 3.6 per cent of lactose, 1 per cent of peptone, and 10 grams of calcium carbonate to 300 c. c. of the solution was inoculated with this organism and put in the ice box. After 38 days the lactic acid isolated was found to be active in form (0.1303 gram of zinc lactate gave 13.04 per cent of water of crystallization); hence it is clear that low temperature does not change the direction of the reaction.

The foregoing experiments lead to the conclusion that the *B. lactis acidi* examined or its enzym, either in the presence or absence of antiseptics, is not the direct cause of the disappearance of active lactic acid and the appearance of racemic acid. Probably the same conclusion is applicable to cheese curd.

## ACTION OF OTHER GROUPS OF BACTERIA

Consideration must now be given to organisms other than *Bacterium lactis acidi* as an explanation of the change in optical activity of lactic acid in cheese.

A yellow Micrococcus was isolated from cheese and inoculated into a lactose solution containing 5 per cent of lactose, 1 per cent of peptone, and 10 grams of $CaCO_3$ to 300 c. c. of solution. After 48 hours of incubation, toluol was added. According to analyses made 72, 82, and 105 days after adding the toluol, the quantity of lactose remained constant.

Therefore, the yellow coccus, in this case at least, had no enzymic action on lactose in the presence of toluol. No lactic acid could be isolated from media similar to the above, inoculated with the same coccus, and incubated without antiseptics. This is further corroborative of the fact that the coccus group, as a group, is not a lactic-acid producer and consequently could have no large part in the lactic-acid changes observed in the curds.

In order to ascertain whether the presence of the Micrococcus has some influence on *Bacterium lactis acidi* in the latter's action on milk sugar, a mixture of the two bacteria was inoculated into a lactose solution containing peptone and calcium carbonate. The results show that active acid was produced, but not racemic acid, as 3.45 grams of zinc-lactate crystals were obtained, containing 12.96 per cent of water of crystallization. It should, however, be remembered that in this group were found two strains (Tables VIII and IX) which could produce lactic acid on milk media. From the above experiment, where a Micrococcus was inoculated alone into a lactose solution, no lactic acid was obtained, but when this organism, presumably the same one, was inoculated into milk a quantity of lactic acid was produced. The ether extract from 300 c. c. of milk which had been inoculated with the organism gave over 100 c. c. of N/10 acidity. Upon neutralizing with $Ba(OH)_2$ a voluminous precipitate occurred. The filtrate from this precipitate required about 40 c. c. of N/10 $ZnSO_4$ solution to take up the barium present. The solution of zinc salts was evaporated to a small quantity and allowed to stand in an ice box for crystallization. There was obtained 0.1031 gram of crystals, which contained 17.53 per cent of water of crystallization. As this is very close to the theoretical percentage of water of crystallization in racemic zinc lactate (18.18 per cent), very nearly all of the lactic acid thus formed was racemic. It would be easy to infer that the organisms from the coccus group discussed above were able to produce very different end-products with some variation in the nature of the media. It is, however, more than probable that the organisms dealt with were distinct in type and physiological action and that the second coccus discussed was one of the strains capable of producing lactic acid.

The next organisms investigated with respect to forms of lactic acid produced were those which belong to the *Bacterium casei* group and which produce a much higher acidity than *B. lactis acidi*, although they grow more slowly than the latter. Several solutions of sterile milk and calcium carbonate inoculated with a culture of *B. casei* gave, on incubation, levo-lactic acid, although Heinemann (1909), experimenting in the same direction with members of this group, obtained racemic acid. Another culture which was isolated from milk gave dextro acid instead of the levo form. These were evidently two different varieties of *B. casei*. They will be designated "*Bacterium casei* 1" and "*Bacterium casei* 2."

*Bacterium casei* 2 was inoculated into 250 c. c. of sterile milk. Calcium carbonate was added, and the medium was allowed to stand for 7½ months. The liquid of the medium had almost evaporated at the end of that time, and crystals had deposited on the bottom of the flask. These crystals were purified by repeated crystallization and then dried in the desiccator. In the resulting product was found 18.07 per cent of calcium, the theoretical percentage of calcium being 18.34 for anhydrous calcium lactate. Rough isolation of lactic acid gave about 0.900 gram of zinc-lactate crystals. Fractional crystallation proved that it was active, the salt being levo-rotatory—that is, the free acid was dextro-rotatory.

Pure cultures of *Bacterium lactis acidi* and *B. casei* and mixtures of these two cultures with and without calcium carbonate were examined, to determine the types of lactic acid present.

Lactic acid was isolated. Its zinc salt was fractionally crystallized, and the water of crystallization was estimated in each fraction of crystals. See Table XXII.

TABLE XXII.—*Analysis of 300 c. c. of sterilized milk, showing production of lactic acid by Bacterium lactis acidi and Bacterium casei 1*

| Fraction No. | I. Bacterium lactis acidi. | | II. Bacterium lactis acidi and B. casei 1. | | III. Bacterium lactis acidi and B. casei 1 with calcium carbonate. | | IV. Bacterium casei 1. | |
|---|---|---|---|---|---|---|---|---|
| | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. |
| | *Grams.* | *Per cent.* | *Grams.* | *Per cent.* | *Grams.* | *Per cent.* | *Grams.* | *Per cent.* |
| 1 | 1.66 | ..... | 1.47 | 18.06 | 4.40 | 18.36 | 2.54 | 12.87 |
| 2 | .73 | 13.36 | .92 | 17.25 | 3.30 | 12.75 | .16 | 12.88 |
| 3 | ..... | ..... | .06 | 17.95 | 3.00 | 13.24 | .16 | 12.89 |
| 4 | ..... | ..... | ..... | ..... | 3.00 | 12.90 | ..... | ..... |
| 5 | ..... | ..... | ..... | ..... | .34 | 12.26 | ..... | ..... |
| 6 | ..... | ..... | ..... | ..... | .65 | 11.98 | ..... | ..... |
| | Levo-salt. | | | | Dextro-salt. | | Dextro-salt. | |

*Bacterium lactis acidi* produced levo zinc lactate, as has already been shown, and *B. casei* 1 gave dextro zinc lactate. The mixture of the two gave racemic acid, as shown in section II. It may be that in curing cheese after pressing, factors similar to those used in these experiments are operative in the production of the racemic variety of lactic acid.

The data in section III, which were obtained from the mixed culture of *Bacterium lactis acidi* and *B. casei* containing calcium carbonate, show racemic zinc salt and also active acid, the latter being produced, no doubt, by *B. casei* 1, the activity of which continues after that of *Bacterium lactis acidi.*

Another experiment, with *Bacterium casei* cultures 1, 2, and 3, the last-named being a pure culture supposedly of a different type from 1 and 2, was carried out as in the previous experiments. The results are given in Table XXIII.

TABLE XXIII.—*Analysis of medium consisting of 200 c. c. of sterile milk and 6 grams of calcium carbonate, showing the production of lactic acid from Bacterium casei 1, 2, and 3*

| Fraction No. | Culture 2. | | Cultures 1 and 2. | | Culture 3. | |
|---|---|---|---|---|---|---|
| | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. | Zinc lactate. | Water of crystallization. |
| | *Grams.* | *Per cent.* | *Grams.* | *Per cent.* | *Grams.* | *Per cent.* |
| 1 | 3.40 | 12.91 | 13.42 | 18.00 | 2.12 | 12.94 |
| 2 | .32 | 13.03 | .71 | 16.14 | 4.19 | 15.12 |
| 3 | .28 | 13.00 | .64 | 14.09 | .00 | ..... |
| 4 | .00 | ..... | .00 | ..... | ..... | ..... |
| | Levo-salt. | | Dextro-salt. | | Dextro-salt. | |

The data show that the mixture of *Bacterium casei* 1 and 2, which in pure cultures produce the two different active lactic acids, gives racemic acid with a slight excess of levo-acid produced by culture 1. This phenomenon might also take place in cheese ripening, producing racemic acid. *Bacterium casei* 3 produces dextro zinc lactate just as culture 1 does, and it also produces the same kinds of volatile fatty acids. Therefore, culture 3 may have been identical with culture 1.

From the foregoing experiments it may at least be concluded tentatively that the formation of racemic lactic acid in Cheddar cheese soon after going to press is due to the later development of organisms of the *Bacterium casei* group principally, together with the possibility that certain forms of the coccus groups can likewise produce racemic lactic acid.

## SUMMARY

(1) Representatives of the coccus groups of organisms isolated from Cheddar cheese when grown in milk produced large quantities of the volatile acids, particularly acetic acid. These acids were produced from

citric acid or lactose or protein, as the medium was practically free from fat. These organisms did not produce formic acid. As they are present at times in very large numbers in cheese, they, no doubt, produce much of the volatile fatty acids which arise during the ripening process.

(2) One of the strains of Streptococcus b was found to produce comparatively large quantities of alcohols and esters—bodies which contribute in a large degree to the flavor of cheese.

(3) A dilute solution of acetic acid and alcohol formed esters by mere contact, without bacterial action. In cheese, however, the dilution is probably too great for this manner of ester formation.

(4) Lactic acid was generally not formed by the coccus groups.

(5) The representatives of the *Bacterium casei* group examined gave results differing from those obtained from the coccus forms. They produced no formic acid, but did form some propionic and much acetic acid.

(6) These organisms produced a large quantity of lactic acid, both active and racemic, and decomposed the citric acid of the media.

(7) Cheese made from chloroformed fresh milk did not yield any volatile fatty acids, showing that inherent milk enzyms are not capable of producing these bodies in any appreciable quantity.

(8) Representatives of both the coccus and *Bacterium casei* groups were able to produce ammonia from milk.

(9) Whey and fresh curds contained active lactic acid. Cheese 1 day old contained a mixture of active and racemic lactic acids.

(10) The cause of the disappearance of active lactic acid and the appearance of racemic acid may be due to enzymic action, combined with the action of those bacteria which can produce both kinds of acid.

(11) Some representatives of the *Bacterium casei* group produced levo lactic acid and others dextro lactic acid from milk. A mixture of these two varieties produced racemic lactic acid. A mixture of *B. lactis acidi* and a levo-producing member of the *B. casei* group gave racemic and active lactic acid. The active acid was probably the result of the longer continued activity of *B. casei*.

(12) Racemic lactic acid found in curing cheese may therefore be produced in a small degree by enzym action, but more probably by the combined action of *Bacterium lactis acidi* and the organisms of the *B. casei* group.

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# CITRUS-ROOT NEMATODE

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## INTRODUCTION

Our ignorance concerning nematodes in general and soil-inhabiting nematodes in particular is well illustrated by the history of the Citrus-root parasite *Tylenchulus semipenetrans*, which, within a few months of its discovery in California, has been located in such widely separated places as Florida, Spain, Malta, Palestine, and Australia. This series of events is, the writer believes, simply illustrative of the surprises in store whenever the soil-inhabiting nematodes receive at the hands of agricultural scientists the attention they merit.

Nematodes are distributed far and wide in inconceivable numbers [1] and without doubt constitute a group in the animal kingdom comparable with insects both in number of species and economic importance.

## THE CITRUS-ROOT NEMATODE TYLENCHUS SEMIPENETRANS

The anatomical features of *Tylenchulus semipenetrans* [2] are so well set forth in the accompanying illustrations that it is unnecessary to describe them further. It is well to add that the drawings are so carefully made [3] that many features set forth in them can not be seen in the natural object, except with the aid of the best immersion lenses skillfully used under favorable conditions.

*Tylenchulus semipenetrans* (fig. 1) was first discovered in California on Citrus roots by J. R. Hodges, Horticultural Inspector for Covina County, Cal., and was first carefully studied by E. E. Thomas, of the Agricultural Station of the University of California, at Berkeley.[4]

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[2] Cobb, N. A. Notes on Monnochus and Tylenchulus. *In* Jour. Wash. Acad. Sci., v. 3, no. 10, p. 288, 1913. This article contains the writer's diagnosis of the new genus Tylenchulus and its type species, *T. semipenetrans*, Cobb.

[3] The drawings were prepared under the author's direction by Mr. W. E. Chambers, of the Bureau of Plant Industry.

[4] Thomas, E. E., A preliminary report of a nematode observed on Citrus roots and its possible relation with the mottled appearance of Citrus trees. Cal. Agr. Exp. Sta. Circ. 85, 14 p., 8 fig., 1913 (J. R. Hodges mentioned).

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Since that time the writer has given some attention to the anatomy, life history, and distribution of this species, and in cooperation with the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, and its correspondents abroad, notably the American consuls stationed in Citrus-growing regions, has shown that it is probably world-wide in its distribution. Samples of the feeding roots of Citrus trees, such as Tylenchulus infests (figs. 2, 3), were sent to these various foreign correspondents, with the request that they forward to Washington from their localities similar roots taken from trees that appeared to be suffering from malnutrition—trees that were off color. These roots on being received at the Department were examined, with the result that this Citrus parasite was found to infest Citrus roots at Valencia, Spain, and at Malta. Previously, the nematode had been found in small numbers in one locality in Florida. Through the courtesy of Mr. Charles O. Chambers, of Gosford, New South Wales, Australia, roots of Citrus were obtained from his locality. These proved also to be Tylenchulus-infested. Similarly, roots of Citrus sent from Haifa, Palestine, through the courtesy of Mr. Aaron Aaronsohn, were found infested. Particular mention should be made of the numerous specimens of roots of Citrus and soil received from Mr. R. S. Vaile, Horticultural Commissioner for Ventura County, Cal., as well as from Messrs. J. W. McLane and R. L. Piemeisel, of the Bureau of Plant Industry. The evidence shows this parasite to occur in widely separated parts of the world, and it probably occurs in every region where Citrus trees have been successfully grown for any considerable length of time.

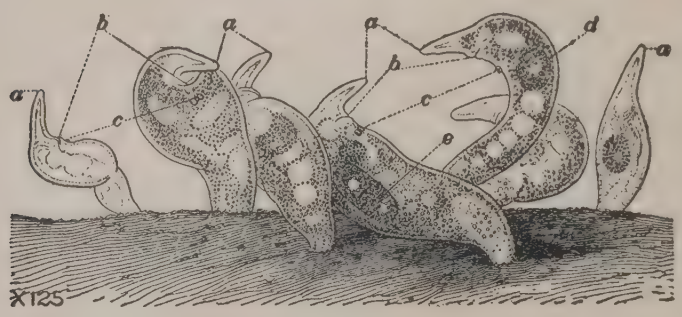



FIG. 1.—*Tylenchulus semipenetrans:* Mature and half-grown females, with their head ends permanently embedded in the feeding root of a citrus tree. This is a plant parasite similar in many ways to the notorious gallworm *Heterodera radicicola*. *a*, Tail end; *b*, vulva; *c*, excretory pore; *d*, *e*, egg in uterus.




FIG. 2.—Healthy Citrus root magnified 5 diameters. Note the size and form of the healthy root endings. Compare with figure 3.

It appears also that *Tylenchulus semipenetrans* is peculiar to the feeding roots of Citrus trees. It has never been found attacking any other species of roots, although careful search has been made for it on a variety

of plants from Florida and also a variety of plants collected in the vicinity of Citrus groves in California by Drs. L. J. Briggs and H. L. Shantz, of the Bureau of Plant Industry, both of whom have taken an active and exceedingly helpful interest in the nature of the Tylenchulus disease, on account of its possible bearing on malnutrition of Citrus trees, a subject to which they are giving special attention. Hundreds of samples of roots of a great variety of plants other than Citrus, collected from various parts of the world, have been examined by the writer without disclosing any specimens of Tylenchulus.




FIG. 3.—Citrus root attacked by the parasitic nematode *Tylenchulus semipenetrans*, magnified 5 diameters. The parasites are shown at *a*. They are shown black, but in reality are yellowish or brownish. Compare with figure 2, and note that owing to the presence of the parasite the feeding roots may become somewhat enlarged and irregular and that the outside portion of the root is somewhat separated from the axial portion, as shown at *b*. When the roots are agitated in water, the outside portion sometimes becomes loosened in segments which will slide on the axial portion *b*, somewhat as beads slide on a string.

We may take it as fairly well established that *Tylenchulus semipenetrans* is a parasite peculiar to Citrus roots, occurring in all parts of the world where Citrus trees have long been grown.

In searching for *Tylenchulus semipenetrans* care must be taken not to confuse it with other species of nematodes. Fortunately its characters are so very well marked that there is very little difficulty in establishing its identity if adult females can be found (see fig. 1). On the other hand, unfortunately, its larval forms closely resemble those of certain other species of nematodes—so closely, indeed, that they can not be identified with certainty, except with the aid of an oil-immersion lens skillfully used by a person conversant with the characteristics of the various genera and species of nematodes. Much the same may be said of the males, as these also are rather exceptionally hard to identify with certainty. The fitness of these remarks will appear when it is stated that already the writer's examinations, directed primarily at *Tylenchulus semipenetrans*, have revealed the presence about the roots of Citrus trees in various parts of the world of toward 100 different species of nematodes, many of which are spear-bearing and have larval forms which might easily be confused with those of Tylenchulus by one unskilled in the art of identifying nematodes species.

Whenever larval nematodes from near roots of Citrus are being examined with the object of ascertaining whether or not they belong to the species *Tylenchulus semipenetrans*, the observer should keep in mind that

the development of the male of Tylenchulus is of a somewhat peculiar character, in that as it increases in age, it may decrease somewhat in size, and that the oral spear, so characteristic a mark of the group of genera to which Tylenchulus belongs, deteriorates so markedly in this instance that adult males are sometimes found that appear to have no trace whatever of this organ. In all cases it deteriorates so much as to fade into an inconspicuous feature of the anatomy (fig. 4). It is somewhat doubtful whether the males enter the unimpaired tissues of Citrus roots. In fact, most of the evidence appears to point the other way and seems to indicate that they seldom, if ever, enter sound roots. The males mature rapidly, and there is some doubt whether they feed at all; for, as already stated, instead of increasing in size as they grow older, they decrease and become more slender. They are probably ill-fitted to bore their way into the tissues of Citrus roots, lacking, as they apparently do, an efficient puncturing organ. All specimens of Tylenchulus seen by the writer to be embedded in comparatively sound roots have proved to be females, though it is entirely possible that an examination of a larger number of cases might prove that males also embed themselves.

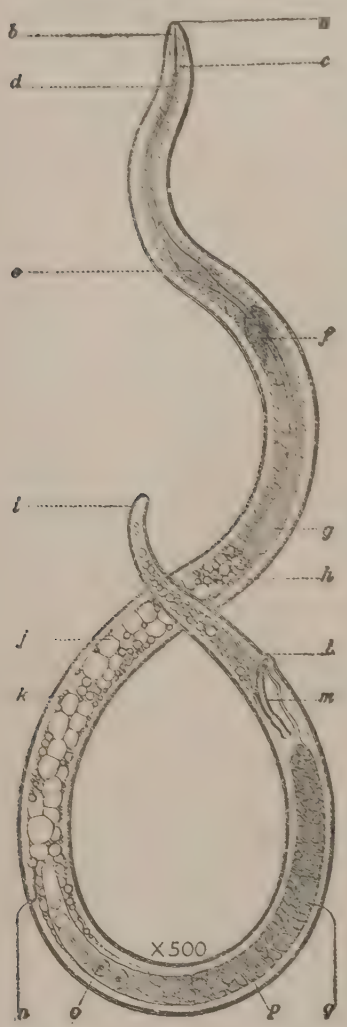



FIG. 4.—*Tylenchulus semipenetrans*: Lateral view of full-grown male. The spear is usually very inconspicuous—always deteriorated. Note also the deteriorated median bulb, sometimes apparently absent. In the male, in contrast with the female, the anus develops. *a*, Lip region; *b*, spear; *c*, 3-bulbed base of spear; *d*, œsophageal lumen; *e*, median œsophageal bulb; *f*, nerve ring; *g*, cardiac œsophageal bulb; *h*, beginning of the intestine; *j*, large intestinal granule; *k*, small intestinal granule; *l*, anus; *m*, spicula; *n*, excretory pore; *o*, spermatocyte; *p*, vas deferens; *q*, spermatozoon.

The eggs of *Tylenchulus semipenetrans* are of comparatively large size, thin-shelled, and usually are not deposited until after segmentation begins. Their size is such that the uterus of the adult female commonly contains only one or two at a time (figs. 1 and 5); and, as a rule, these are found in the early stages of segmentation, sometimes containing only one blastomere, sometimes two, sometimes three, occasionally as many as a dozen. The exact length of time the eggs remain in the uterus is at present unknown, but under favorable conditions does not exceed a few days. They are deposited one at a time in batches of a dozen to a score or more and are sometimes found encased in a somewhat indefinite mass of "gummy" matter. Judging from the experiments, these eggs hatch very promptly, probably within a day or two after being deposited, and produce colorless

larvæ of the form shown in figures 6 and 7. The movements of these larvæ are slow and weak, and yet the young stages, especially those of the female, are more active than those of the adult. At no stage of its existence are the movements of *Tylenchulus semipenetrans* anything but relatively slow and weak, and it is altogether improbable that through its own muscular exertions it ever migrates any great distance. Once the head of the female becomes well embedded in a Citrus root, in the manner shown in figure 8, *c*, it is practically impossible for her to retreat. She therefore becomes fixed for life and dies at the point where this entrance was made. "Shells," or empty skins, of dead females are not infrequently found on infested roots. Figure 8, *c*, shows a female that has partly penetrated a Citrus root. It will be seen that at the surface of the root the body is constricted, both the portion inside and that outside having a much greater diameter (see also fig. 9). It is the swollen character of the anterior portion of the embedded female that prevents retreat. As she grows older and increases in size, her head penetrates farther and farther, but never so far as to be out of harmony with the specific name "*semipenetrans*." By the use of her strongly developed oral spear, the tissues of the roots are punctured, and the food, consisting of the sap and protoplasmic matter of the root, is ingested.




FIG. 5.—*Tylenchulus semipenetrans*: Female ready for fertilization. Note the increased size of the excretory pore, *n*, as compared with previous stages; see figures 7 and 12. The pore is located farther back than in any other known species of nematode. *a*, Lip region; *b*, guide of spear; *c*, shaft of spear; *d*, lumen of œsophagus; *e*, median œsophageal bulb; *f*, nerve ring; *g*, cardiac œsophageal bulb; *h*, beginning of intestine; *o'*, ovary; *n'*, duct of renette; *n*, excretory pore; *r*, vulva; *s*, wings of the cuticle; *l'*, deteriorated anus; *i*, terminus.

The females of *Tylenchulus semipenetrans* sometimes appear to be somewhat gregarious; at least it is not uncommon to find the adult females arranged in rather definite groups on the roots. The cause of this phenomenon is not yet understood but may, perhaps, arise in this way: Larvæ from the same batch of eggs in searching for food naturally seek the nearest suitable root. The mother worms, having injured or killed the root in their immediate vicinity and the root having accord-

ingly thrown out a new shoot farther back, as often happens in such cases, the new generation of young females make their way to this new shoot. This supposition accords with what is known about the growth of roots in general when injured by disease-producing organisms and is in harmony with the weak muscular powers of the *Tylenchulus*.

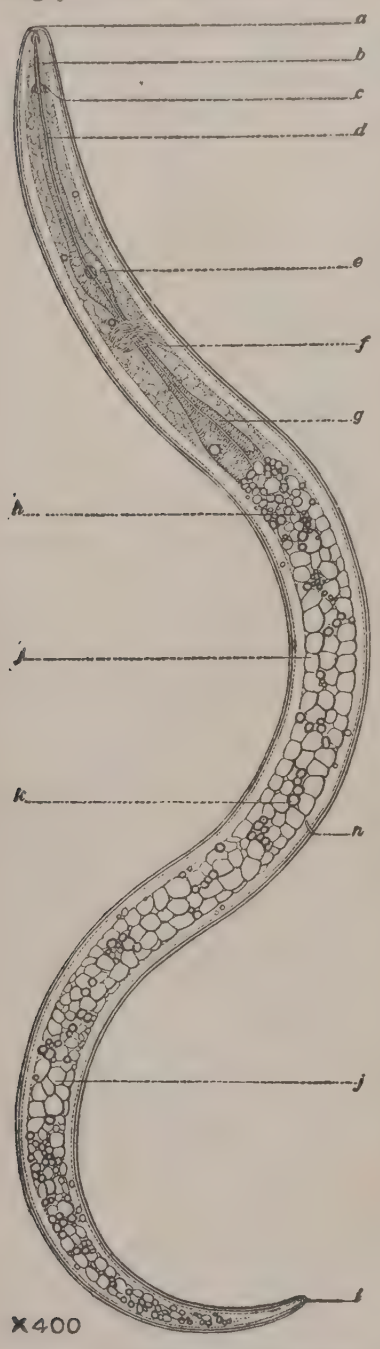



FIG. 6.—*Tylenchulus semipenetrans:* Larva, soon after hatching from the egg. Note the absence of the anus. *a*, Lip region; *b*, spear; *c*, 3-bulbed base of spear; *d*, lumen of the œsophagus; *e*, median œsophageal bulb; *f*, nerve ring; *g*, posterior œsophageal bulb; *h*, beginning of the intestine; *j*, large intestinal granule; *k*, small intestinal granule; *n*, excretory pore; *i*, terminus.

Although the movements of *Tylenchulus semipenetrans* are so feeble as to make it seem quite unlikely that it travels any great distance by the aid of its own muscular powers, its small size is in favor of its transportation from place to place in the soil by a great variety of agencies, such as soil water, subterranean insects, worms, and burrowing animals. In this way it may be carried to considerable depths in the soil and doubtless will be found attacking Citrus roots, however deep the latter penetrate. When cultural operations bring *Tylenchulus semipenetrans* to the surface and these cultural operations are followed by irrigation, the eggs and free-living young stages may be carried from tree to tree in the same grove by the irrigation water, or from orchard to orchard, or even occasionally from district to district; in fact, wherever Citrus fruits are grown under irrigation, the irrigation water is undoubtedly one of the principal agents in distributing the pest after it has once become established through the planting out of infested nursery stock.

*Tylenchulus semipenetrans* is comparatively sensitive to temperatures much above those of ordinary soil. When eggs, larvæ, or adults are placed in water above 100° F., they are quickly affected, and at 130° F. are killed. Immersion in water at 140° F. causes almost instant death to all forms of the organism. This fact was demonstrated and utilized in studies of the life history.

To follow the development of the eggs and larvæ, they were placed in capsules constructed in accordance with the explanation accompanying

figures 10 and 11. In the incubation experiments it was thought advisable to give the eggs and developing larvæ conditions as nearly as possible like those in the vicinity of growing Citrus roots. In order that uncertainty might not creep into the experiments, it was only necessary that the material to be placed in the small glass brood capsules be Pasteurized at a temperature of 130° to 140° F. This was done with entire success. Lest any misapprehension arise at this point, it is well to state that not all species of soil-inhabiting nematodes are so sensitive to high temperatures. It is therefore best to use for incubating material soils as free as possible from other species of nematodes.

The form of the brood capsule devised for the incubation experiments is illustrated in figure 11. These glass capsules are easily constructed and manipulated, and, as said before, the hatching and rearing offer few difficulties. The drawing of the capsule is to scale and the explanation is comparatively complete, so that nothing need be added except to say that the material surrounding the egg in the capsule consists of soil particles and Citrus-root detritus, both taken from the immediate vicinity of diseased roots of Citrus. This material, having been Pasteurized, as before described, was used as a nidus in hatching Tylenchulus eggs and rearing the larvæ. The capsule was half filled with the nidus, and then the egg to be incubated was introduced by means of a very fine-pointed pipette. As this operation was accomplished with the aid of a magnifying glass of one-half inch equivalent focus, there was proper assurance that no other nematode organism was introduced. The remainder of the nidus was then inserted above the egg and the disk *f* introduced. Afterwards the disk *e* was placed in position. Necessarily, considerable water was intro-

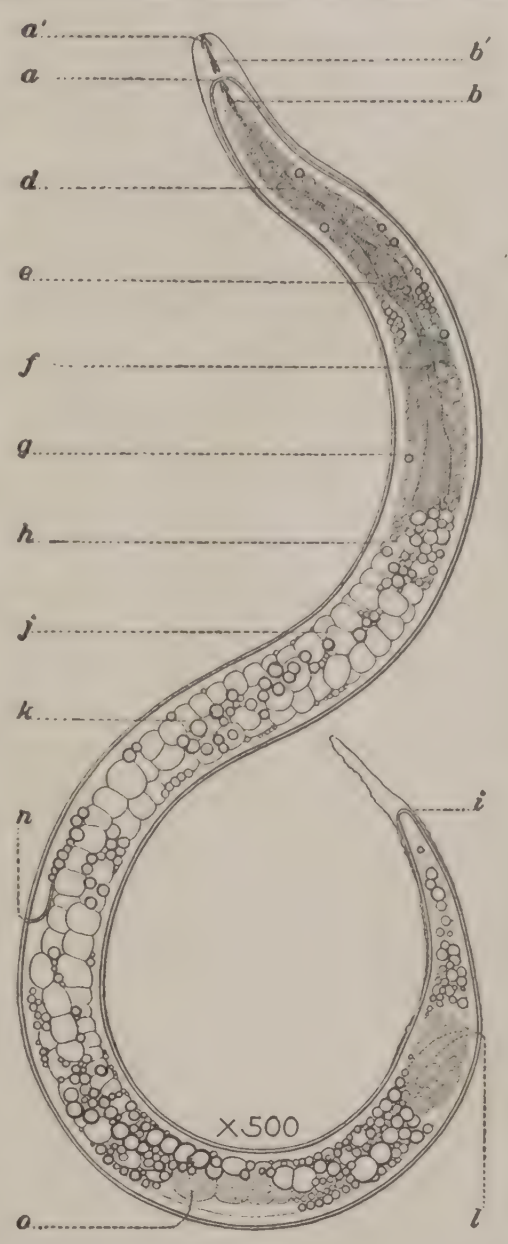



FIG. 7.—*Tylenchulus semipenetrans:* Young larva of male undergoing the first or second molt. Note the deteriorating spear and median bulb, indicating that the males are not so well equipped to penetrate the Citrus roots as the females. The males mature rapldiy, perhaps with little or no food. *a*, Lip region; *a'*, lip region of sloughed skin; *b*, deteriorating spear; *b'*, spear of the sloughed skin; *d*, lumen of the œsophagus; *e*, median œsophageal bulb; *f*, nerve ring; *g*, cardiac œsophageal bulb; *h*, beginning of the intestine; *j*, large intestinal granule; *k*, small intestinal granule; *n*, excretory pore; *o*, immature internal sexual organ; *l*, developing male sexual opening; *i*, terminus.

duced during these operations. Therefore, before the capsule was buried in the soil, it was placed on blotting paper, so that as much water as possible might drain away. This left the capsule in a condition to reabsorb moisture. The soil about the potted Citrus seedling to be used in the experiment was previously allowed to become somewhat dry; in fact, was allowed to go without water until the seedling showed the first signs of distress. The prepared capsule was then placed in the pot adjacent to sound young Citrus feeding roots and the soil replaced in the pot (see fig. 10). The Citrus seedling was then at once watered, so that the capsule was given an immediate opportunity to absorb soil moisture that might be described as "of a citrous character."

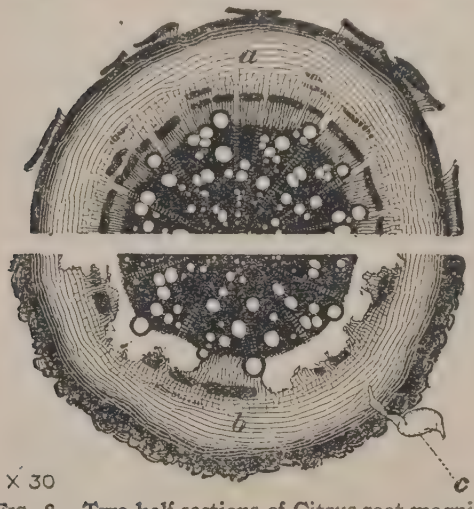



FIG. 8.—Two half-sections of Citrus root magnified 30 diameters: *a*, Healthy root; *b*, diseased root. A female specimen of *Tylenchulus semipentrans* is shown at *c* with her head end embedded in the root. Note that the outside portion of the diseased root, shown light, is nearly detached from the central axial portion, shown black. Compare with figure 3.

In the spring of 1913 a number of experiments were started with a view to ascertaining the length of time required for the larvæ of *Tylenchulus semipenetrans* to become adult. For this purpose potted grape-fruit seedlings of a size and character illustrated in figure 10 were used. Material derived from Tylenchulus-infested Citrus roots was added to the water supplied to the seedlings. In most cases some of the surface soil was removed, and the infested water applied several days in succession in such a way that there could be no doubt that an abundance of the living larvæ had come in contact with the roots. Previous examinations indicated that few, if any, of the female larvæ added to the water were advanced beyond the earliest stages shown in the illustrations. Check plants were subjected to the same treatment as the infested plants, with the exception that no material containing *T. semipenetrans* was added to the water. There was no difficulty in rearing

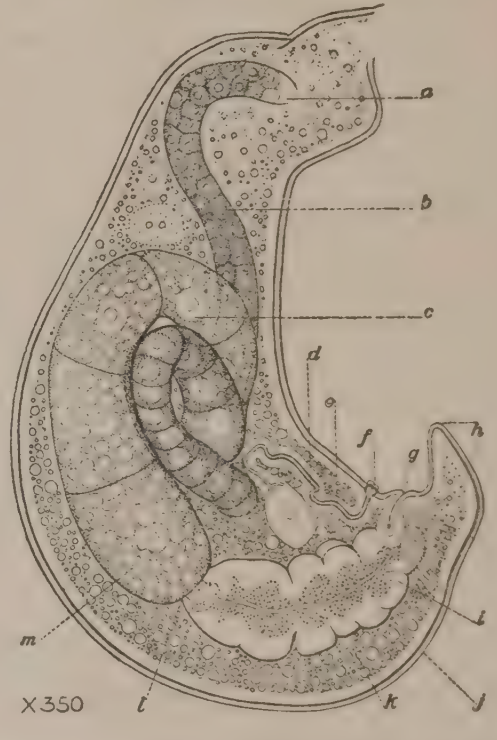



FIG. 9.—*Tylenchulus semipenetrans:* Posterior portion of adult female. The portion shown is located outside of the Citrus root. Compare figures 1, 3, 8, and 13. *a*, Blind end of the coiled single ovary; *b*, anterior portion of ovary; *c*, posterior portion of ovary; *d*, renette cell; *e*, duct of the renette cell; *f*, excretory pore; *g*, vulva; *h*, terminus; *i*, uterus; *j*, cuticle; *k*, somatic granule; *l*, junction of ovary with uterus; *m*, posterior end of ovary with ovum ready to enter uterus.

Tylenchulus by his simple process, and in from six to seven weeks it was possible to secure adult females from roots thus infested.

The following is a typical case: On May 31, 1913, adult females, as well as larvæ and eggs, were removed from the roots of a grapefruit seedling first infested on April 14, 1913. As a rule, the full-grown males were found much earlier than the females; in fact, there can be little doubt that in most cases the infested water supplied at the beginning of the experiment contained a few males that were adult or nearly so. The gist of these experiments is contained in the fact that the infested water would contain but few, if any, females that were advanced beyond the stages shown in figures 12 and 13, and that the entire life cycle of the females under the conditions of the experiments was shown to be accomplished in six to eight weeks.




FIG. 10.—Pot containing Citrus seedling. A portion of the wall of the pot has been removed to show location of one of the glass brood capsules shown in figure 11. *a*, Brood capsule; *b*, label of same attached to capsule by means of a thread which passes down through the soil. Tylenchulus eggs incubated in this way often hatch out in 24 hours. Larvæ reared on grapefruit roots in pots similar to that shown above become adult in six to seven weeks.

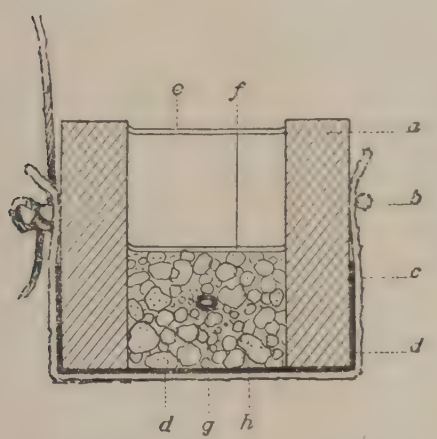



FIG. 11.—Longitudinal section of a glass brood capsule enlarged 10 diameters. The cord at the left leads upward to the surface of the soil. Compare with figure 10. In these capsules the youngest eggs of *Tylenchulus semipenetrans*, even those removed from the uterus, hatch out in 24 to 72 hours when the capsules are placed adjacent to healthy roots of growing Citrus seedlings. *a*, glass tube shown in longitudinal section; *b*, thread encircling capsule, shown in cross section; *c*, finest mesh linen cloth held over bottom of filter capsule by the encircling cord *b*; *d*, layer of filter paper, for convenience shown black; *e*, *f*, disks of filter paper; *g*, nematode egg embedded in Pasteurized orange root detritus and soil granules.

A few words about the probable origin of *Tylenchulus semipenetrans* may not be out of place. As this parasite occurs in so many different, very widely separated Citrus regions and is found only on Citrus roots, it is a fair supposition, as before remarked, that it is peculiar to species of Citrus. The most reasonable explanation of its wide distribution is that it has been sent from point to point in commerce on the roots of Citrus nursery stock. As this distribution of the parasite has probably been going on for centuries, the obvious surmise is that the original habitat of the parasite is that of the genus Citrus itself.

The original home of the cultivated species of Citrus is supposed to be India or southern China, and we may therefore suppose these lands to be also the original habitat of the Tylenchulus.

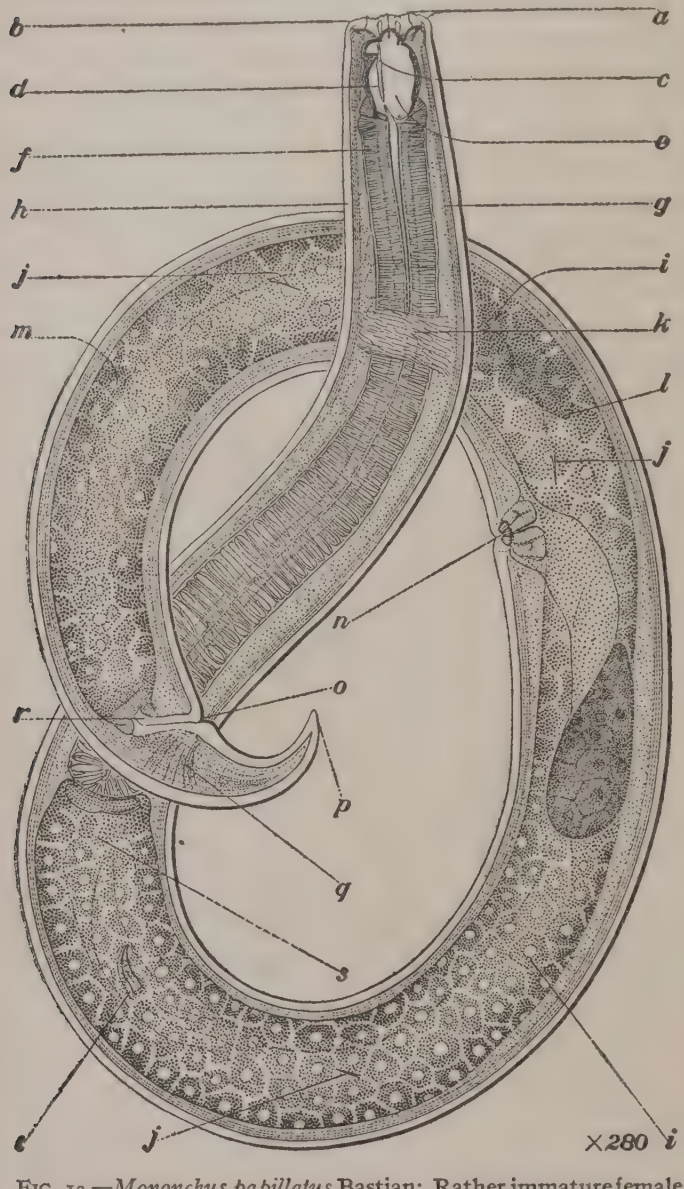



FIG. 12.—*Mononchus papillatus* Bastian: Rather immature female specimen which has been feeding upon *Tylenchulus semipenetrans*. The remains of three or four Tylenchuli are to be seen in the intestine. *a*, Two of the innervated papillæ existing on one of the six mobile lips; *b*, one of the lips; *c*, dorsal pharyngeal tooth; *d*, one of the three longitudinal chitinous ribs of the pharynx; *e*, pharyngeal cavity; *f*, œsophagus; *g*, muscular layer of the body; *h*, cuticle; *i*, one of the cells of the intestine; *j*, *j*, *j*, oral spears of three ingested Tylenchuli; the spear in the intestine near the vulva is accompanied by an undigested portion of the lumenof the œsophagus of the Tylenchulus; *k*, nerve ring; *l*, blind end of the anterior ovary, which, being behind the intestine, shows less clearly than the posterior ovary; *m*, nucleus of one of the intestinal cells; *n*, vulva; *o*, anus; *p*, terminus; *q*, anal muscles; *r*, rectum; *s*, cardia; *t*, spicula of an ingested male Tylenchulus. The outlines of the undigested tail end of the male are to be seen faintly.

The life history of *Tylenchulus semipenetrans* strikingly illustrates how little we know about organisms inhabiting the soil. Here is an organism so large as to be visible to the naked eye, an organism that, considered as a microscopic object, is relatively huge, occurring, practically speaking, in most parts of the world where Citrus fruits can be profitably grown, attacking and killing the roots of a crop of great economic importance. Yet this organism remained undiscovered for 200 years after mankind possessed instruments for its discovery. With such a striking instance before us, how can we doubt that thoroughgoing biological soil researches will reveal a multitude of similar cases? It is a common observation among agriculturists that crops fail for some unknown cause. Such cases may appear less mysterious when we have an adequate knowledge of soil-inhabiting organisms.

Not the least interesting feature of these investigations into the life history of *Tylenchulus semipenetrans* has been the discovery of a species of *Mononchus*, another nematode, which regularly feeds upon the *Tylenchulus*, swallowing the males and the larvæ whole. Conclu-

sive evidence on this point is the presence in the intestine of individuals of *Mononchus* of the remains of specimens of *Tylenchulus*. In several such cases no other ingested food appeared to be present, showing that the *Mononchus* had been making a meal on *Tylenchulus*. In searching for the remains of species of Tylenchulus in the intestine of a specimen of Mononchus, one looks for the more indigestible parts, such as the oral spear and the spicula of the male (see figs. 4 and 6). These being very indigestible and also highly refractive remain visible even when other parts of the Tylenchulus have been completely digested.

This Mononchus is a relatively large species and, judging from this fact alone, would seem to be capable of devouring *Tylenchulus semipenetrans* in considerable numbers. These discoveries confirm those made earlier by the writer and render it certain that there is a class of beneficial nematodes inhabiting the soil.

The pharynx of the active and predacious Mononchus is supplied with a prominent acute, dorsal, forward-pointing chitinous tooth. This tooth is opposed to six thick, muscular, hookshaped, backacting lips, and it is by the interaction of these various organs that the Tylenchulus is seized, punctured, and killed. Possibly the acute pharyngeal tooth of the Mononchus is nothing less than a poison fang, but this at present is only a matter of theory. It is, however, known that many nematodes have glands in the segments of the œsophagus—the so-called œsophageal glands—and it is possible that these glands may secrete a poisonous liquid substance which may be used in the same way as the venom of serpents. Furthermore, the inner surface of the pharynx of the Mononchus is sometimes armed with scores of exceedingly minute rasplike teeth, which in some respects resemble, on a small scale, those found in the pharynx of serpents and sharks, teeth which aid

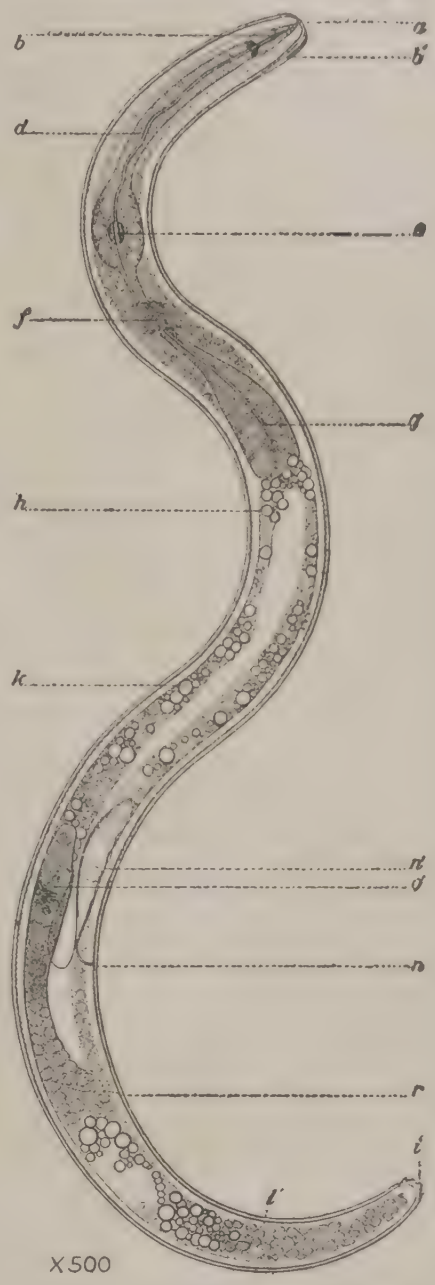



FIG. 13.—*Tylenchulus semipenetrans:* Young female about to undergo its second molt. Compare with figures 1 and 6. Note that the spear in the growing female does not deteriorate. Note also the increasing size of the median or suction bulb of the œsophagus. Quite the contrary occurs in the male. *a*, Lip region; *b*, spear; *b'*, spear which has been sloughed off; *d*, lumen of the œsophagus; *e*, median œsophageal bulb; *f*, nerve ring; *g*, cardiac œsophageal bulb; *h*, beginning of the intestine; *k*, larger intestinal granules; *n*, excretory pore; *n'*, renette cell; *o'*, blind end of single immature ovary; *r*, developing uterus; *l'*, remnant of rudimentary anus; *i*, terminus.

these latter in swallowing organisms of relatively large size. It is a natural supposition that the minute teeth in the pharynx of the Mononchus serve a similar purpose. These minute teeth do not occur in the Mononchus here mentioned as feeding on Tylenchulus.

The writer has in his possession a specimen of Mononchus caught in the act of swallowing another nematode. The Mononchus holds its prey in the grip of its "jaws" and partially swallowed. The phenomenon reminds one of the not infrequent discovery of serpents with half-swallowed birds in their maws. The writer has also observed aquatic species of Mononchus whose intestines contained only the remains of rotifers, indicating that the species of Mononchus do not feed exclusively upon other nematodes.

These definite observations on the Mononchus lend new interest to the study of this genus, which is estimated to contain a very considerable number of species, probably a hundred or more when all shall have been enumerated. Upward of 20 species are known to the writer. The examination of almost any good collection of soil-inhabiting or fresh-water nematodes is pretty sure at the present date to reveal one or more hitherto unknown species of Mononchus. Both on the ground of the number of species of Mononchus and the number of their individuals the matter is one well worthy of further observation.

There are other nematode genera that on structural grounds may now be suspected to be vermivorous, or at any rate carnivorous.

## CONCLUSIONS

There can be no doubt that *Tylenchulus semipenetrans* is an injurious parasite. There is conclusive evidence that it kills the feeding roots of Citrus trees. The roots die either as a direct result of the attack of this parasite or of the attack of other organisms following in its wake; in other words, the nematode is a primary cause of the death of the feeding roots. Many cases have come under observation in which it was apparent that, had it not been for the nematode, the roots would have remained in a healthy condition. The evidence along these lines is of the same character as that which is relied on in demonstrating injuries due to insects and other macroscopic parasites.

The extent of the damage which may be properly charged up against this parasite is a different matter, and it will be necessary to collect evidence along this line for several years before a final statement can be made. Up to the present the data obtained indicate unquestionably that the investigations should be continued.

The writer's long experience with numerous fungous diseases of the Citrus family has led him to conclude that, though a few of these diseases are very harmful, most of them are of minor importance. There are many fungous diseases of the bark, foliage, and fruit of Citrus trees of such a character that they are easily controlled. In many cases all that is

needed is the careful application of well-known suitable cultural methods. Where the climate, soil, and cultural conditions are suitable to the Citrus family these diseases will not appear, or will appear in such a mild form as to be largely negligible. An examination of the literature of Citrus diseases and of the fungi and other microorganisms which have been found parasitic upon Citrus trees discloses a long list of parasites, so long, indeed, as to suggest that species of Citrus are liable to the attacks of an unusually large number of parasitic microorganisms. The foregoing remarks, of course, apply only to aerial parts of the tree. If, therefore, among these numerous parasites of the above-ground portion of the tree, relatively few are found that are really harmful, the thought arises that something similar may be true of the subterranean parasites. Should this prove true and should *Tylenchulus semipenetrans* prove to be one of a series of rather harmless parasites which attack the roots of these trees, the injurious results of which may be combated by proper cultural methods, then all may be well. This, however, is something that needs to be demonstrated. The history of this parasite is altogether too recent and incomplete to render final judgment possible. Hence the necessity for several years' further careful observation.

It will have been noticed that the evidence thus far accumulated is, in a considerable number of cases, indecisive. It may be compared to a two-edged sword, which may cut in either direction. The parasite is found to be somewhat more abundant in orchards that are out of condition, but so far as the present evidence goes, this may be either because the parasite is there and causing the difficulty, or because the trees are out of condition for some other reason, and therefore are not resistant to the parasite. On the other hand, the parasite is found on the roots of trees which appear to be in good health. Here, again, this may be either because the parasite has not yet been there sufficiently long to cause visible injury or because healthy trees do not have much difficulty in counteracting the bad effects undoubtedly due to the parasite. It would, however, be unwise for Citrus growers to allow such reasoning to lull them into a feeling of security. History abounds with similar cases in which either apathy or optimism has led to deplorable inaction, permitting devastating diseases to continue on their course unchecked.

*Tylenchulus semipenetrans* is distributed from place to place on nursery stock. This is the method by which in all probability it is most frequently distributed from one district to another, and it follows that any method of inspection by which infested nursery stock can be readily distinguished from noninfested will make it possible to establish at least one efficient check on the spread of the disease.

As *Tylenchulus semipenetrans* is a parasite with which from now on Citrus growers will have to reckon, it seems certain that sooner or later appropriate inspection will be undertaken by Federal authorities, by State authorities, and by private enterprise. The most beneficent inspec-

tion is that which results in prevention. To illustrate this point the present conditions in the State of Florida may be cited. An examination of Florida orchards seems to indicate that the parasite is not yet widely distributed in that State; in fact, it has been found in only one locality. If henceforth all nursery stock in Florida is examined for *T. semipenetrans* and no stock is accepted for shipment or planted out unless upon inspection it is found free from this nematode, very much can probably be done to limit the spread of disease.

Hitherto no information has come to light that shows any particular kind of Citrus stock to be more resistant than others. The Tylenchulus has been found to occur upon the following stocks: Sour orange, sweet orange, grapefruit, and *Citrus trifoliata*. Investigations are under way with the object of ascertaining whether these stocks vary among themselves in resistance to the Tylenchulus and whether it is possible to discover a thoroughly resistant stock.

During the life-history studies it was discovered that hot water is fatal to *Tylenchulus semipenetrans*, and that Citrus roots survive the temperature required to kill the Tylenchulus. Some of the smaller roots may be injured, but the larger roots are not seriously harmed, and when the treated trees are planted out, they proceed to grow new feeding roots. These facts lead to the hope that a hot-water treatment may prove to be a more or less efficient means of disinfection or at least prove to be a palliative.

# PRELIMINARY AND MINOR PAPERS

## PELLICULARIA KOLEROGA ON COFFEE IN PORTO RICO

By G. L. Fawcett,

*Plant Pathologist, Porto Rico Agricultural Experiment Station*

In a recent publication Dr. J. Kuijper (1912)[1] states that from a comparison of the fungus causing the Zilverdraadziekte of Surinam and the leaf-blight of coffee (*Coffea* spp.) in Porto Rico he is of the opinion that it is not identical with *Pellicularia koleroga* Cooke of India, judging from descriptions of that fungus, and that it also differs from the fungus causing the candelillo of Venezuela. This conclusion with regard to the identity of the Porto Rican fungus would seem but reasonable if the possession of a gelatinous matrix, such as has been ascribed to *P. koleroga* in some of the descriptions, were necessary to make it that fungus. However, the fact that errors were made in the original descriptions of other coffee fungi and that in other respects the descriptions agreed fairly well with the appearance of the Porto Rican coffee-blight fungus seemed to justify the writer's referring to it as *P. koleroga* in one of the reports of the Porto Rico Experiment Station (Fawcett, 1911). Moreover, specimens of *P. koleroga* which had been collected in Mysore, where Cooke's original specimens were obtained, kindly sent to me by Mr. E. J. Butler, of the Agricultural Institute of Pusa, India, agree in every way with the Porto Rican leaf-blight fungus. It would seem from this that it is a mistake to assume that the Porto Rican fungus was not *P. koleroga* Cooke.

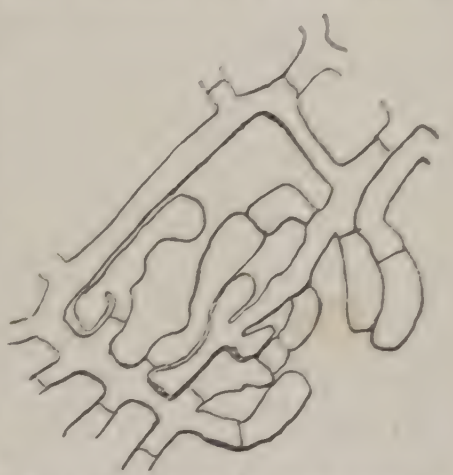


FIG. 1.—Early stage in development of group of hold-fast cells of *Pellicularia koleroga*.

As to the Venezuelan fungus, studies by the writer cause him to agree with Dr. Kuijper that it is different from the Porto Rican fungus. It is this difference, however, which shows that it is not *Pellicularia koleroga*. The candelillo of Venezuela is of especial interest, in that Dr. Cooke (1881) identified specimens sent to him at Kew as *P. koleroga*, as he considered the coffee leaf-blight of the Old and New Worlds to be the same, and consequently all the publications on coffee diseases that have since appeared have similarly treated the subject. This view is in the main correct, but only accidentally so, since, apparently, the Venezuelan candelillo is caused by a related but quite distinct fungus. The appearance of the affected trees, characterized principally by blackened leaves hanging from fungous threads, is the same. But the affected leaves in specimens

[1] Bibliographic citations in parentheses refer to "Literature cited," p. —.

gathered in the spring of 1913 were found to be somewhat different, one of the principal differences being the absence of the finely mottled appearance which is taken on at one stage of the disease by leaves affected with *P. koleroga*. This mottling is caused by aggregations of hold-fast cells (fig. 1). In the South American specimens studied such cells are much more evenly distributed and when gathered form in smaller, closer groups.



FIG. 2.—Appressoria of Venezuelan candelillo fungus, from an old, fully developed specimen.

A further difference is that instead of consisting of numerous crowded and, in the older ones, overlapping branches originating from various parts of the surrounding mycelium, the hold-fast cells of the Venezuelan candelillo are made up of rather small expansions from isolated short side branches (fig. 2). These fasten the hyphæ to the leaf in much the same way as the appressoria of the powdery mildews, to which, however, they bear but slight resemblance, except in arrangement along the hyphæ and in function. Still, the earlier classification of the candelillo by Dr. Adolf Ernst (1878, p. 16) as one of the Erysiphaceæ seems less ill-founded if this and not *P. koleroga*, as has been sometimes assumed, were the fungus in question.

The manner of branching and size of the hyphæ are the same, but the Venezuelan fungus possesses somewhat thinner or, at least, less conspicuous cell walls, and in places masses of unbranched threads running in all directions are to be found, which are rarely found in the Porto Rican fungus. The differences are such as might be found in closely related species of the same genus. Although the specimens studied were gathered during the dry season and for that reason were not in the best condition, they serve to show that some small but real differences exist between the candelillo of Venezuela and the leaf-blight of India and Porto Rico. In the original description *Pellicularia koleroga* is described as possessing spores, hyaline, echinulate, of about the same diameter as the hyphæ, in which they lie without apparent connection. The fact that spores are lacking in the Porto Rican fungus has been taken as additional evidence that it is not *Pellicularia koleroga*. This, however, is a point of little importance. On one leaf of the Indian specimens examined, spores were found which agree with the original description (fig. 3). They had no connection with the larger hyphæ, but were seen to be attached to very fine hyphæ belonging, appar-

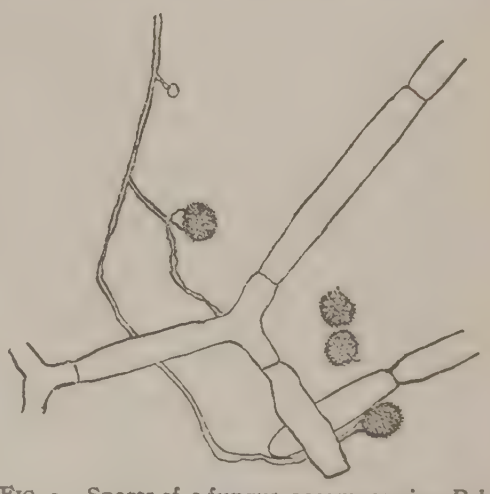


FIG. 3.—Spores of a fungus accompanying *Pellicularia koleroga*.

ently, to some fungus other than *Pellicularia koleroga*—possibly to some of the saprophytes by which it is sometimes accompanied. In some of the Venezuelan specimens, spores occurred which answered fairly closely to the descriptions. Later, an Aspergillus was found on some of the leaves which had clearly produced spores on the candelillo-affected leaf. It seems probable that the spores described as belonging to *Pellicularia koleroga* are really those of some other fungus, so that the absence of spores in the species of Surinam and Porto Rico has no bearing on the identity of the fungus, but merely means that the saprophyte producing such spores is not present.

In brief, *Pellicularia koleroga* of India occurs in the Antilles and also on the mainland of South America. Another fungus of similar habit, causing the so-called candelillo, is also found on the continent and is apparently the only fungus of this nature found in those regions of Venezuela in which were collected the first specimens identified, apparently erroneously, as *Pellicularia koleroga* Cooke.

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# FEEDING HABITS OF THE BOLL WEEVIL ON PLANTS OTHER THAN COTTON

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In the course of the investigations on the biology of *Anthonomus grandis* at Victoria, Tex., during the summer of 1913, under the direction of Mr. W. D. Hunter, the writer was able to conduct a number of experiments on the possibility of the boll weevil's breeding in some of the native malvaceous plants. Since the results secured differ with the plants, they are grouped under the various species of plants tested.

The nutritive value of these plants is best shown by a comparison of the longevity of boll weevils fed upon them and the length of life of specimens fed upon cotton and also those kept without food. For this reason the following summary taken from experiments conducted at the same time is given. Forty boll weevils placed on moist sand immediately after emergence and left without food gave a maximum longevity of 6 days, the average for the two sexes being 3.3 days. A number of boll weevils fed only on cotton bolls gave a maximum longevity of 32 days and an average of 17.2 days. Those fed only on cotton leaves had a maximum life of 45 days and an average of 12 days. Of course, the boll weevils fed on cotton squares lived longer than any others. Their maximum life period was 74 days, the average being 40 days.

## FEEDING EXPERIMENTS WITH SPHAERALCEA LINDHEIMERI

*Sphaeralcea lindheimeri* Gray is found in small groups on some of the sandy areas near Victoria, though it is comparatively rare. This is evidently the northern part of its range. It is a low-growing, crown-branching plant, and is extremely tomentose throughout. The petals in the buds are very loosely packed and are tightly covered by a heavy, woolly calyx. The buds are very poorly adapted either for the feeding or breeding of boll weevils.

Early in the season six hibernated individuals were collected from cotton in the field and placed with buds, blooms, and fruit of *Sphaeralcea lindheimeri*. These boll weevils fed quite readily, but deposited no eggs. In 22 daily examinations failure to feed was noticed on 5 days. The feeding was never very extensive and was usually confined to the corolla.

The life of these boll weevils after being placed on Sphaeralcea was rather short, especially when the amount of feeding is considered. The maximum longevity was 15 days, and the average of both sexes was 8.5 days. It is quite probable that the boll weevils would have been able to live almost as long without any food whatever. The average life of a number of boll weevils collected in the field about the same time and fed on cotton squares was 46.2 days.

Later in the season another experiment was conducted in which boll weevils that had just emerged from cotton squares were placed with the buds, blooms, and fruit of *Sphaeralcea lindheimeri*. There was more

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or less feeding in this series almost every day, but it was practically confined to the blooms only.

Twenty boll weevils, ten of each sex, were used in this series, and their longevity was quite regular, ranging from 2 to 8 days, with an average of 4.2 days. This is not quite 1 day above the average for unfed boll weevils; consequently the nourishing power of the plant was not very high. In fact, it is quite doubtful whether the feeding in either series prolonged the life of the boll weevils in the least.

It is hardly probable that the boll weevil will be able to breed in the buds of this plant. The extremely heavy, woolly calyx renders oviposition very difficult, and the contents of a bud are not likely to be sufficient to nourish a boll-weevil larva to pupation.

## FEEDING EXPERIMENTS WITH CALLIRRHOE INVOLUCRATA

*Callirrhoe involucrata* Gray is quite common in many parts of Victoria County. In fact, it is the most abundant species of the plants studied during the summer of 1913. Since this plant blooms in the early spring and stops about the first week in June, it was impossible to conduct more than one series of experiments with it as food. Fourteen hibernated boll weevils collected from cotton in the field early in the spring were used. Practically all these boll weevils fed freely on the buds and blooms. Owing to the fact that more boll weevils were introduced later in some of the series, very little accurate information can be given regarding their longevity. The maximum certain longevity was 20 days.

A number of boll weevils were observed in copulation in this series. Two females deposited eggs, one laying two and the other three. These five eggs were placed in four buds. The buds were then placed on moist sand and tested for emergence of adults. Since none emerged, the buds were opened and examined. Three showed no signs of larval work, but one showed that a larva had lived long enough to consume fully one-half of the inner tissue of the bud.

## FEEDING EXPERIMENTS WITH CALLIRRHOE PEDATA

*Callirrhoe pedata* Gray is much like the preceding species, but is erect in growth instead of procumbent. It is comparatively rare near Victoria. Rather thorough tests were made of this plant as a food plant for boll weevils. Early in the spring eight hibernated individuals were collected in the field and placed on it. These weevils fed freely on the buds and blooms, but deposited no eggs.

In this series the maximum longevity after collection was 26 days, and the average, 12.1 days. This is considerably above the record of the field-collected boll weevils fed on Sphaeralcea, but is still far short of the longevity of the boll weevils fed on cotton squares.

Later in the season 24 boll weevils which emerged from cotton squares were placed with the buds and blooms of *Callirrhoe pedata* as food. The longevity record of this series is rather surprising. With the exception of one boll weevil, which lived for 21 days and ate regularly every day, the maximum longevity was 6 days, and the average was about as low as that for unfed boll weevils. In the case of the one exception the sustaining value of the plant is shown clearly, but for some reason the remaining 23 boll weevils were not so well adapted to the food, though they ate heartily during the few days they lived. Including the boll weevil which

lived for 21 days, the average longevity was 4.4 days. In this series no eggs were deposited.

This species of mallow probably ranks about the same as *Callirrhoe involucrata* as a host plant for the boll weevil. The buds are smaller, and consequently the chance of breeding is very slight. Both the buds and blooms seem to be of some nutritive value for the boll weevil.

## FEEDING EXPERIMENTS WITH HIBISCUS SYRIACUS

*Hibiscus syriacus* L. is a large, woody perennial, commonly called "white althea." Quite a number of the plants were found growing in lawns and cemeteries throughout Victoria. Several cultural varieties are found, the chief differences being in the color and form of the bloom. The color varies from pure white, through pink to blue and purple. The most important difference, however, is in the arrangement of the stamens and petals. The latter vary from a single row to a great number very irregularly arranged.

The buds are covered with the tough pilose calyx until they begin to open. Superficially a section cut through a bud shows the interior tissues to be much the same as in cotton squares. There is the same arrangement of the petals and immature anthers.

The foliage is very tough, being so different from the tender, succulent foliage of cotton that the boll weevil could not be expected to feed upon it.

Attention was first attracted to *Hibiscus syriacus* by the fact that on June 16 the writer found a boll weevil feeding on the anthers of a bloom at Victoria. The plant was a large one in the rear of the laboratory and stood about 30 feet from a small patch of cotton which was rather heavily infested with boll weevils. When found, the boll weevil was busily eating the pollen of the bloom and had destroyed almost all of the anthers. Since this was the first record of the species being found feeding on any plant except cotton and Thurberia (Arizona wild cotton), it was considered advisable to make thorough tests of the longevity of the boll weevil on *H. syriacus*, and also to determine whether they would breed in the buds. The experiments with this aim may be divided into three series, according to the locality from which the boll weevils were derived.

## EXPERIMENTS WITH TEXAS BOLL WEEVILS

The first series consisted of Texas boll weevils (*Anthonomus grandis*) either collected in the field or reared from cotton squares in the laboratory at Victoria. Different lots were tested on buds alone, blooms alone, and on buds, blooms, and young fruit together.

In order to test the exact nutritive value of buds alone, one series of 10 boll weevils was started on buds alone. The results from this series were very surprising. Feeding was noted on only 2 days, and the maximum longevity was 5 days, with an average for both sexes of 3.7 days. This length of life is very little above that for unfed weevils, and it is extremely doubtful whether the buds prolonged the life of any of the boll weevils in the least. This is quite in accord with the fact that in all series offering a choice of food there was very little feeding on the buds.

Owing to the fact that in the feeding series where a choice of food was offered the boll weevil fed so very much more on the blooms than any other part of the plant, another experiment was conducted to determine the length of life of boll weevils fed only on blooms from the time of their emergence. Six insects were used, and they fed every day from the

starting of the experiment to the death of the last boll weevil. The longevity was surprisingly great, only one boll weevil dying in less than 24 days, and the average for both sexes being 25.3 days, with a maximum of 40 days. It is evident that the blooms are better food than the buds. The longevity of the bloom-fed boll weevils is much greater than of those fed either on cotton bolls or leaves and compares well with the longevity on squares.

The pollen is the first choice of the boll weevils. One weevil will soon destroy every anther in a large bloom and usually emerges covered with pollen. However, in practically every case there is more or less feeding on the corolla itself. This frequently takes the form of large areas eaten from a beginning on the margin of a petal, but often the petal is merely riddled with small holes.

By far the greatest number of experiments on feeding *Hibiscus syriacus* were series where buds, blooms, and young fruit were offered to the boll weevils every day.

Some boll weevils were reared in the laboratory and placed on Hibiscus immediately after emergence, while others were collected in the field and consequently had fed first on cotton.

Three lots of boll weevils collected in the field—12 in all—were used. In two lots they were collected in the field, brought to the laboratory, and were immediately placed in tumblers with a base of moist sand and containing fresh buds, blooms, and young fruit of *Hibiscus syriacus*. In the other lot, hibernated individuals that had been collected some days previously and fed on cotton squares until the time of starting the experiment were used in the same manner. The four pairs collected in the field were in copulation at the time of capture. When possible, the food was changed often enough to give a constant supply of fresh buds, blooms, and young fruit.

The boll weevils all began feeding immediately after being placed with the Hibiscus. In a total of 53 examinations feeding was found in all but 2 cases. Both of these were found toward the last of a series when only one boll weevil remained, affording striking evidence of the readiness with which they fed on Hibiscus even when accustomed to cotton.

An analysis of feeding by the parts of the plant attacked gives the following: Corolla, 40 times; stamens, 40 times; buds, 14 times; and young fruit, 6 times. This shows the very decided preference for the bloom.

Although the females used in two series were in copulation when collected in the field, only one egg was secured during the experiment. This egg was deposited normally in a bud 31 days after the female had been placed on Hibiscus. It hatched, and the larva lived until about half grown. During its life it consumed much of the tissue of the bud.

The maximum longevity was 36 days, the average being 16 days. While this longevity is short when compared with that on cotton, it certainly shows that it is possible for the boll weevil accustomed to feeding on cotton to subsist for a long time on Hibiscus.

Three series of boll weevils reared in the laboratory were used in another experiment—one lot in the spring, one in summer, and one in the fall. In all of these experiments the boll weevils were reared on cotton bolls or squares in the laboratory. They were then placed immediately in tumblers containing a layer of moist sand and offered a mixture of buds, blooms, and young fruit every day until the supply of food was exhausted.

The 12 weevils in the spring series fed quite freely and regularly. In a total of 69 examinations feeding was found in all but 3 cases, and 1 of these was when nothing but mature fruit was offered. An analysis of the feeding shows the following: Corolla, 39 times; stamens, 37 times; buds, 27 times; and young fruit, 10 times. While this shows the usual preference for the corolla and stamens, the amount of feeding on buds is unusually large.

Eggs were deposited by each lot of boll weevils, but on only four different days, a total of 15 being found. The maximum number per lot in one day was eight.

The maximum longevity of these boll weevils was 43 days, with an average of 19.2 days. This is above the average for boll weevils fed on either cotton bolls or leaves.

The period from emergence to deposition was 5 days in each series.

Although 15 eggs were deposited in this series, they were distributed in only 4 buds. These were placed in cloth-covered tumblers on moist sand and tested for emergence of adults. When no adults appeared at the proper time, the buds were opened and the contents examined. Of course, it was impossible to determine at that time whether the eggs actually hatched, but if they did, the larvæ died before reaching any considerable size, as there were no signs of larval work in any of the buds.

Only six boll weevils, divided into three lots, were used in the summer series. In a total of 37 examinations feeding was found in all but 8 cases. The feeding by parts of the plant was divided as follows: Anthers, 24 times; corolla, 13 times; and buds, once.

No eggs were found any time, but owing to the extremely dry weather, the buds at this season were not very choice and the supply was not sufficient to have fresh ones always present.

The maximum longevity for the series was 26 days, the average for the two sexes being 14.1 days.

Twelve boll weevils were started in the fall series on September 9 and 10. These were fed on the buds, bloom, and young fruit of the pink variety of Hibiscus, the food being renewed often enough to insure the presence of a fresh supply all the time. This was continued as long as the food was available.

Four females and seven males were used, one male having escaped on the second day of the experiment. Although these boll weevils were not examined more than once a day, each female was observed in copulation at least once at the time of this examination, seven acts of copulation by the four females being observed.

Each of the females deposited at least one egg, the four depositing 19 eggs. The period from emergence to deposition ranged from 12 to 18 days, with an average of 14 days. The period of oviposition varied from 7 to 15 days, excluding the record of one female that deposited only one egg. The average was 11 days.

In a total of 62 examinations for feeding during the period when food was present the feeding was usually quite extensive, and not a single case occurred when there was no feeding. An analysis by parts of the plant attacked gives the following: Stamens, 52 times; corolla, 50 times; buds, 5 times; and pistil, once.

On October 13 the supply of Hibiscus was completely exhausted, and the boll weevils died 4 to 5 days afterwards, the average being 4.4 days.

As a majority of the boll weevils were still alive at the time it was necessary to stop feeding them, no definite longevity can be given, but the following facts will show something of what might be expected. Of the 11 weevils tested, 1 was accidentally killed when 32 days old, 3 died with an average longevity of 30 days, and 7 were still alive at the time of closing the series—35 days after their emergence. From this it is readily seen that the longevity would have been very great had it been possible to continue the series to the normal death of the boll weevils.

All of the 19 eggs deposited were placed in buds, except 1, which was deposited on the inside of the base of a petal during a day when no buds were fed—September 21. This egg was left on the petal, covered with moist cloth, and placed on moist sand. It hatched on September 25—four days later. The larva appeared completely normal. A fresh Hibiscus bud was opened to the center with a knife, and the small larva was dropped into a cavity formed there. Then the bud was closed and placed on moist sand. This larva was watched by opening the bud every few days. Unfortunately, it became infested with mites (probably *Pediculoides* sp.) when nearly fully grown—October 3. On October 5 it pupated, but died soon after completing the change. The death was probably due to the attack of the mites, as larvæ in immature stages being reared on cotton squares on the same shelf were killed by them.

The remaining 18 eggs were distributed in 11 buds. Two of these produced adult boll weevils, 4 bloomed and thus prevented breeding, 4 showed no signs of larval work, and 1 gave indications of the larva being alive until it had consumed most of the tissue of the bud.

The two adults that emerged were males. In one case the egg was deposited on September 24, the adult emerging on October 12. In the other the egg was deposited on September 27, the adult emerging on October 14—developmental periods of 18 and 17 days, respectively.

A summary of the spring, summer, and fall series of observations is of interest, in that it shows the conduct of the boll weevils throughout the season when offered their choice of all edible parts of the plant. Table I gives the results of the observations on the preference of the boll weevil for certain parts of Hibiscus. It is readily seen that the bloom (stamens and corolla) is very much preferred to all other parts, forming 83 per cent of the total number of times of feeding. That the feeding is quite constant is shown by the fact that in 168 examinations only 11 records of no feeding were made—only 6.5 per cent.

TABLE I.—*Summary of feeding experiments of the Texas boll weevil, showing its preference for certain parts of Hibiscus syriacus*

| Series. | Part of plant. | | | | |
|---|---|---|---|---|---|
| | Stamens. | Corolla. | Bud. | Fruit. | Pistil. |
| Spring.....times fed.. | 37 | 39 | 27 | 10 | 0 |
| Summer.....do.... | 24 | 13 | 1 | 0 | 0 |
| Fall.....do.... | 52 | 50 | 5 | 0 | 1 |
| Total..... | 113 | 102 | 33 | 10 | 1 |

The longevity will be discussed in the general summary. Eggs were deposited in only two series, 34 being found.

### EXPERIMENTS WITH LOUISIANA BOLL WEEVILS

In order to determine whether the feeding habits on *Hibiscus syriacus* of boll weevils reared from cotton at Victoria, Tex., were adaptive habits acquired by long presence there and whether they were peculiar to that locality, a number of cotton squares infested with the same species were imported from Tallulah, La., and the adults reared from them were also tested for feeding and longevity on Hibiscus. As blooms only were available, no other food was offered. The four boll weevils used fed very readily on the blooms and were able to subsist on them for very long periods. At the time of closing the experiment, owing to the lack of food, only one out of the four weevils had died, and this one had lived for 21 days. The remainder were still alive and feeding at this time—33 days after starting the first lot and 32 days after the second.

In the 39 examinations recorded, absence of feeding was found only once. The anthers were attacked 32 times and the corolla 18 times.

It is greatly to be regretted that a supply of buds was not available so that tests could have been made of the breeding of these Louisiana boll weevils in them, as the lack of either positive or negative records on this point make the results less definite than they would otherwise have been. But the readiness with which these boll weevils fed on the blooms and their extreme longevity seem to indicate that they are quite as well adapted to Hibiscus as those from Texas and quite as likely to breed in it.

The importance of this plant as food for boll weevils is shown by the fact that the three remaining died in an average of 4.6 days after the last day of feeding.

### FEEDING EXPERIMENTS WITH ANTHONOMUS GRANDIS THURBERIAE

Since *Anthonomus grandis*, var. *thurberiae*, was already adapted to at least one plant other than cotton (*Thurberia thespesiodes*), it was considered probable that it would be able to breed in Hibiscus buds. For determining this point a number of these boll weevils which had just emerged from Thurberia bolls imported from Arizona were placed on Hibiscus. The results of these experiments follow.

A few boll weevils emerging from Thurberia bolls were placed with blooms of Hibiscus and tested for the longevity and feeding. A number of boll weevils were started in this series, but several lots were killed in the first few days by what seemed to be a bacterial disease. This reduced the number to two, which were carried through the experiment. These boll weevils did not feed nearly so readily nor so much as the Texas or Louisiana boll weevils under observation at the same time. In the total of 21 observations two records of no feeding were made. Feeding on the anthers was noted 18 times and on the corolla, 13 times.

One boll weevil lived 15, the other 30 days, an average of 22.5 days. The number tested was too small for this record to be of much value.

In the second series nine weevils were fed on the buds, blooms, and fruit of Hibiscus—five females and four males. In the 40 observations made while food was present there was not a single case of no feeding. The analysis of the feeding shows parts of the plant fed on, as follows: Corolla, 31 times; stamens, 31 times; and buds, 15 times. The usual preference for the blooms is shown.

This series was also interrupted by exhaustion of the food supply. Of course, this prevents a definite statement of their longevity, but the fol-

lowing summary will give an idea of what might have been expected from these weevils. Of the nine boll weevils started, two died with an average longevity of 6 days, while seven were still alive when the food became exhausted—34 days after the emergence. These seven weevils died on an average of 4.8 days after the stoppage of feeding.

Each female was observed in copulation at least once, and a total of 13 records of copulation were made in the course of the daily examination. The first pair observed in copulation performed this act 6 days after emergence.

Thirty-six eggs were deposited, eighteen in each series. The periods from emergence to oviposition were 12 and 14 days in the cases where this record was available.

The eggs deposited were placed in 21 buds, an average of 1.7 eggs per bud. Three of these buds produced adult boll weevils, 8 bloomed, 9 showed no sign of larval work, and 1 was nearly consumed by a larva before its death. The eggs deposited on September 21, 24, and 30 produced a male, a female, and a male on October 8, 10, and 15—developmental periods of 17, 16, and 15 days, respectively.

## SUMMARY OF HIBISCUS EXPERIMENTS

From the foregoing experiments it is quite evident that it is possible for *Anthonomus grandis* and *A. grandis thurberiae* to breed in the buds of *Hibiscus syriacus*. And not only is this possible, but all indications point toward the conclusion that this breeding would be no rarity. While there was little oviposition and no breeding in the series conducted early in the season, this may have been due to the writer's lack of knowledge of the correct way to keep the food in proper condition. The oviposition in the fall series may seem low, but that of females on squares at this time was no higher. The weather was unusually cold during this period and the oviposition of all boll weevils, regardless of food, was extremely variable by days.

These data prove beyond doubt that the boll weevils fed from the time of emergence only on the buds and bloom of Hibiscus can develop sufficiently sexually to produce a number of normal fertile eggs and to deposit them normally. The copulation of these boll weevils was quite normal. Almost every pair was observed in copulation in the course of the daily examination. In a number of experiments in attempting to get weevils to copulate when they had been fed only on cotton leaves since emergence the writer was successful in only a very limited number of cases and was never able to secure a record of any of these depositing eggs, though they lived for long periods. From this it seems probable that some feeding on bud, bloom, or fruit tissue is necessary for sexual maturity, and the buds or bloom of Hibiscus will serve this purpose instead of those of cotton.

The eggs deposited were all placed in normally sealed punctures, and dissection of the buds always showed them to be placed partially within the inner folds of the corolla and partially within the outer layer of immature anthers. At least 90 per cent of all the eggs deposited were placed in older buds which had started to open slightly, and the punctures were made through the exposed tips of the involuted corolla. The favorite location of these egg punctures is through the corolla in the base of the clefts then forming between the sepals. This deposition in the corolla is probably due to the extreme pilosity and toughness of the calyx.

However, a few eggs have been deposited in punctures made through the calyx, but in these cases the boll weevils seem to experience great difficulty in sealing the opening. The tissue of the exposed corolla probably most nearly approximates that in which the boll weevils are accustomed to develop. It is of interest to note that of the boll weevils collected from cotton in the field and placed on Hibiscus only one deposited a single egg.

The older buds of Hibiscus are very hardy, and the puncture of the boll weevil very rarely prevents them from opening and shedding the eggs or larvæ. The number of instances of this occurring is readily shown by a glance at the preceding records of tests of the eggs. Although these buds had been picked from the plants and placed in tumblers, a very large percentage of them bloomed and so prevented breeding. This characteristic of Hibiscus, taken in connection with the habits of the boll weevil, is undoubtedly of great importance in preventing it from breeding in the buds. The boll weevil naturally selects the older buds, which are slightly opened at the tips, for oviposition, but these buds are usually able to open and rid themselves of the pest.

The food preference displayed by the boll weevil is quite pronounced. Almost all feeding is on the corolla and the stamens of the bloom. Next to these in importance come the buds and then the young fruit. The latter are so very different in tissue and formation that it is not surprising that boll weevils will not feed on them to any extent. In fact, the only cases of feeding on fruit were when it was young, usually within a day or two of the dropping of the bloom.

While the various series of the Louisiana and Texas boll weevils and the Arizona Thurberia weevil were not sufficiently similar to allow an exact comparison, some indication of the extent and nature of the adaptation can be seen. The conduct of the three types of weevils in relation to feeding was practically the same. All showed the same food preference, and, allowing for quite natural variations, the extent of the feeding was much the same. While the experiments with the Louisiana boll weevils were quite limited in extent, they gave all indications of as much adaptation to the food as the native and Thurberia weevils.

The longevity of these weevils is of considerable interest. Since the three series under way when the supply was exhausted were by far the most important in this respect, the only figures which can be given are very unsatisfactory. The average longevity of both sexes feeding on buds alone was 3.7 days. That for blooms alone was 25.3 days. As these two series were carried on at the same time and were identical in conditions, the comparison shows the relative food values of the two. Of the boll weevils fed on buds, blooms, and fruit the spring series averaged 19.2 days and the summer series, 14.1 days. However, these records are shown to be of little value when compared with the fall series. The longevity of the three series, interrupted by the lack of food, may be summarized as follows: Of the 24 weevils started on this food, 17 were still alive, 1 was killed when 32 days old, and 6 had died with an average longevity of 20.5 days. This was on October 13, or 33 days after the emergence of the adults used. Both sexes of the boll weevils collected in the field and then fed on Hibiscus died on an average of 16 days after being placed on this food.

The developmental periods of the boll weevils under discussion are shown in Table II.

TABLE II.—*Comparison of developmental periods of Texas boll weevils and Thurberia weevils in buds of Hibiscus and of Texas boll weevils in cotton*

| Thurberia weevils on Hibiscus. | | | Texas boll weevils on Hibiscus. | | | Developmental period for Texas boll weevils in cotton squares at same time. |
|---|---|---|---|---|---|---|
| Eggs deposited. | Adults emerged. | Developmental period. | Eggs deposited. | Adults emerged. | Developmental period. | |
| | | *Days.* | | | *Days.* | *Days.* |
| September 21..... | October 8... | 17 | September 24. | October 12.. | 18 | ..... |
| September 24..... | October 10.. | 16 | September 27. | October 14.. | 17 | ..... |
| September 30..... | October 15.. | 15 | ..... | ..... | ..... | ..... |
| Average..... | ..... | 16 | ..... | ..... | 17.5 | 17 |

From Table II it is seen that there was little difference between the Thurberia weevils and Texas boll weevils in the Hibiscus buds. The average development period of native Texas boll weevils from eggs deposited in cotton squares during this same time is offered for comparison.

## SUMMARY

Table III shows a summary of the longevity of the various series.

TABLE III.—*Summary of longevity of boll weevils on various Malvaceae tested.*

| Period of experiment. | Food plant. | Series and remarks. | Longevity. | | | |
|---|---|---|---|---|---|---|
| | | | Maximum. | Average. | | |
| | | | | Male. | Female. | Weighted. |
| | | | *Days.* | *Days.* | *Days.* | *Days.* |
| May 13 to May 28. | Sphaeralcea lindheimeri. | Hibernated boll weevils collected in field. | 15 | 7.3 | 10.0 | 8.5 |
| Do. | .....do..... | Boll weevils reared from cotton squares. | 8 | 4.3 | 4.1 | 4.2 |
| May 13 to June 2. | Callirrhoe involucrata. | Boll weevils collected in the field.... | 20 | ..... | ..... | ..... |
| May 16 to June 11. | Callirrhoe pedata..... | .....do..... | 26 | 14.0 | 10.2 | 12.1 |
| June 13 to July 11. | .....do..... | Boll weevils reared from cotton squares. | 21 | 5.3 | 3.6 | 4.4 |
| June 27 to July 1. | Hibiscus syriacus..... | Reared boll weevils fed on buds only. | 5 | 3.2 | 4.2 | 3.7 |
| June 27 to Aug. 5. | .....do..... | Reared boll weevils fed on blooms only. | 40 | 30.0 | 16.0 | 25.3 |
| June 16 to July 22. | .....do..... | Boll weevils collected in the field and fed on buds, blooms, and fruit. | 36 | 15.3 | 16.8 | 16.0 |
| June 15 to July 28. | .....do..... | Boll weevils reared from cotton squares and fed on buds, blooms, and fruit; spring series. | 43 | 15.1 | 23.3 | 19.2 |
| Aug. 28 to Sept. 23. | .....do..... | Boll weevils reared from cotton squares and fed on buds, blooms, and fruit; summer series. | 26 | 9.0 | 19.3 | 14.1 |
| Sept. 5 to Oct. 8. | .....do..... | Anthonomus grandis thurberiae reared from Thurberia bolls and fed on blooms only. | 30 | ..... | 22.5 | 22.5 |

TABLE III.—*Summary of longevity of boll weevils on various Malvaceae tested*—Contd.

| Period of experiment. | Food plant. | Series and remarks. | Longevity. | | | |
|---|---|---|---|---|---|---|
| | | | Maximum. | Average. | | |
| | | | | Male. | Female. | Weighted. |
| | | | *Days.* | *Days.* | *Days.* | *Days.* |
| Sept. 9 to Oct. 18. | Hibiscus syriacus..... | Boll weevils reared from cotton squares and fed on buds, blooms, and fruit; fall series; boll weevils still alive at conclusion of experiment. | 35+ | ..... | ..... | 33.5+ |
| Sept. 1 to Oct. 18. | .....do..... | Boll weevils reared from squares imported from Tallulah, La.; fed on blooms only; still alive at conclusion of experiment. | 33+ | ..... | ..... | 29.7+ |
| Sept. 9 to Oct. 18. | .....do..... | Anthonomus grandis thurberiae reared from Thurberia bolls and fed on buds, blooms, and fruit; still alive at conclusion of experiment. | 34+ | ..... | ..... | 26.6+ |
| June 18 to July 20. | Cotton..... | Bolls only..... | 32 | 24.0 | 12.6 | 17.2 |
| June 9 to July 24. | .....do..... | Leaves only..... | 45 | 15.2 | 8.8 | 12.0 |
| June 5 to Oct. 10. | .....do..... | Squares only..... | 74 | 35.3 | 41.4 | 40.0 |
| June 22 to July 2. | Unfed..... | On moist sand..... | 6 | 3.4 | 3.3 | 3.3 |

The averages of the three unfinished series are included in Table III for comparison. From these it is seen that the record for native Texas boll weevils on Hibiscus is very little short of the final average for cotton-square-fed boll weevils, although the latter were continued to death.

Eggs were deposited in only two plants, *Callirrhoe involucrata* and *Hibiscus syriacus*—5 in the former and 71 in the latter. By series those in Hibiscus were divided as follows:

Field-collected female, 1 egg; spring series, 15 eggs; fall series, 19 eggs; and *Anthonomus grandis thurberiae* series, 36 eggs.

All experiments were performed under cage conditions, but these were made as nearly normal as possible. No boll weevils have been found breeding in plants other than cotton and Thurberia under field conditions, and only one case of feeding under such conditions has been observed. This was in the case of a single boll weevil found feeding on *Hibiscus syriacus*, at Victoria, Tex., on June 16.

# IDENTITY OF PERIDERMIUM FUSIFORME WITH PERIDERMIUM CEREBRUM

By GEORGE G. HEDGCOCK, *Pathologist*, and W. H. LONG, *Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry*

In this paper the Cronartium stage of the fungus under discussion is called *Cronartium cerebrum* (Peck), n. comb., in place of the *C. quercus* (Brond) Arthur or *C. quercuum* Miyabe of previous writers. This new combination is made because the authors, after a careful examination of authentic material of the so-called *Cronartium quercus* of Europe, find that it is not the same fungus as the American Cronartium on oaks (*Quercus* spp.).

*Peridermium cerebrum*, the name for the æcial stage of the American Cronartium on oaks, was published by Peck in 1873,[1] and as this is the oldest specific name for the fungus the combination *Cronartium cerebrum* (Peck) must be used.

Further evidence that *Cronartium quercus* of Europe is not *Cronartium cerebrum* of America is found in the following facts:

(1) Only the uredinial stage of the European fungus has been found. If this rust was the same as the American species, then the telial stage should certainly have been collected, as it follows closely (within 5 to 10 days) the uredinial stage.

(2) No æcial stage corresponding to *Peridermium cerebrum* has been reported from the European countries where the so-called *Cronartium quercus* is found.

An investigation of *Cronartium cerebrum* and of its æcial form (*Peridermium cerebrum* Peck) has been conducted by the senior writer for a number of years. As a result of numerous inoculations of several species of pines (*Pinus* spp.) with the telia of this fungus from pedigreed cultures inserted in wounds and made under control conditions in the greenhouse at Washington, D. C., it has been found that in the pines having two needles, or two to three needles, in the cluster, globoid swellings or galls are usually formed on the limbs and twigs, while in pines having three needles in the cluster, fusiform, spindle-shaped, or oblong swellings are commonly found, which are occasionally accompanied by a reversion to the juvenile type of leaves, and by the formation of hexenbesen (witches'-brooms).

In the inoculation experiments mentioned above spheroid swellings were commonly formed on *Pinus contorta*,[2] *P. edulis*, *P. densiflora*, *P. divaricata*, and *P. virginiana;* and fusiform swellings, as a rule, were formed on *P. coulteri*, *P. ponderosa*, *P. radiata*, and *P. sabiniana*. Since fusiform swellings are produced by *Peridermium fusiforme* Arthur and Kern (Pl. XI, fig. 2) and *Cronartium cerebrum* is found throughout the range of this Peridermium, these results suggested that it might be identical with *Peridermium cerebrum* (Pl. XI, fig. 1).

In nature the writers have observed that the spheroid galls are usually found on *Pinus divaricata*, *P. clausa*, *P. echinata*, *P. glabra*, *P. resinosa*,

[1] Peck, C. H. Descriptions of new species of fungi. In Bul. Buffalo Soc. Nat. Sci., v. 1, p. 68. 1873.
[2] The nomenclature of trees used in this paper is that of Geo. B. Sudworth, U. S. Dept. Agr., Div. Forestry Bul. 14, 1897.

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and *P. virginiana*, and that fusiform swellings occur on *Pinus serotina* and *P. taeda*. The former are commonly produced by *Peridermium cerebrum* and the latter by *Peridermium fusiforme*. In the same locality on pines of the 2-needle group the swellings are spheroid and typical of *Peridermium cerebrum*, and on adjacent pines of the 3-needle group they are usually fusiform and typical of *Peridermium fusiforme*.

The junior writer has observed that the fusiform type of swelling (*Peridermium fusiforme*) on *Pinus taeda* is often accompanied by a marked development of hexenbesen (witches'-brooms) at the distal end of the swelling. At Brooksville, Fla., many trees of *P. taeda* are badly infected with this Peridermium, and in almost every instance the diseased branches terminate in hexenbesen. Occasionally the fusiform swellings are very long, ranging from several inches to over 4 feet in length. The largest swellings are found on the trunks of trees 3 to 6 inches in diameter. Those on the 3-needle pines often originate near the extremity of a branch, and, as the side branches develop, the fungus invades them, producing an enlargement of the base of each branch. In such cases a continuous swelling is formed, extending in both directions on the main branch and to the adjacent side branches.

The junior writer collected on March 6, 1914, sporulating specimens of *Peridermium fusiforme* on *Pinus taeda* (F. P. 15138)[1] near Gainesville, Fla., associated directly with the young leaves of *Quercus nigra*. On March 23 he found *Cronartium cerebrum* on *Q. nigra* (F. P. 15170) and on *Q. phellos* in direct contiguity with *Peridermium fusiforme* on *P. taeda* (F. P. 15177) near Brooksville, Fla. Inoculations were made by the senior writer with the æciospores of the first collection (F. P. 15138) on oaks (*Quercus* spp.) on March 10, 1914, in the pathological greenhouses at Washington, D. C. On April 3 the telia of *C. cerebrum* were present sparsely on the leaves of *Q. rubra* (F. P. 15217) and of *Q. velutina* (F. P. 15218).

Later and more abundant collections of *Peridermium fusiforme* were made by the junior author in Florida, South Carolina, and North Carolina on *Pinus serotina* and *P. taeda*. Inoculations made with æciospores from these on several species of oaks resulted in nearly every instance in the appearance of abundant uredinia of *Cronartium cerebrum* in 7 to 10 days (Pl. XI, fig. 4) and of numerous telia in 15 to 21 days from the time of inoculation. All control plants in every set remained free from infection. The following species of oaks were most abundantly infected with the Cronartium from inoculations made with *Peridermium fusiforme*: *Quercus californica* (F. P. 15316), *Q. digitata* (F. P. 15300), *Q. gambelii* (F. P. 15287), *Q. imbricaria* (F. P. 15286), *Q. lobata* (F. P. 15299), *Q. michauxii* (F. P. 15294), *Q. phellos* (F. P. 15278), and *Q. rubra* (F. P. 15297). The following species of trees were infected less abundantly: *Castanopsis chrysophylla* (F. P. 15334), *Quercus alba* (F. P. 15309), *Q. bicolor* (F. P. 15331), *Q. emoryii* (F. P. 15318), *Q. velutina* (F. P. 15330), and *Q. virginiana* (F. P. 15317).

The uredinia, telia, and sporidia of the Cronartium resulting from inoculations with the æciospores of *Peridermium fusiforme* differ in no essential feature from those obtained by inoculating the same species of oaks with the æciospores of *P. cerebrum;* in fact they can not be distinguished morphologically from the latter (Pl. XI, fig. 3).

[1] The numbers in parentheses refer to specimens in the collections for study in the Office of Investigations in Forest Pathology.

The chief difference between these two species of Peridermium, according to the original description of each, appears to be the formation of globoid swellings on the host tree attacked by *Peridermium cerebrum* (Pl. XI, fig. 1) and of fusiform swellings by *P. fusiforme* (Pl. XI, fig. 2). Both species have the cerebroid arrangement of the æcia, while their æciospores and peridial cells agree closely in size, color, and shape.

From a series of field observations made by the senior writer during the last four years it is established that in case of the swellings of *Peridermium cerebrum* on *Pinus virginiana* the pycnia precede the æcia 12 months, instead of preceding them during the same spring. In other words, the pycnia and æcia occur during alternate years, and two years is the time required for a life cycle of all forms of spores of the rust. Usually only pycnia or only æcia are found on a gall during the same season, but occasionally both are found on different parts of the same swelling. In such cases the part bearing pycnia does not bear æcia till the following spring and vice versa. In no case have both pycnia and æcia been found at the same time, the one succeeding the other during the same season on the same portion of the surface of a swelling.

The junior writer recently noted pycnia on the swellings caused by *Peridermium fusiforme* on pines in Florida. They were noted as occurring on separate swellings from those bearing æcia, indicating the same alternation as in *P. cerebrum*.

The field and cultural data here given prove conclusively that *Peridermium fusiforme* and *P. cerebrum* are both æcial stages of the same fungus, *Cronartium cerebrum*, and are not even sufficiently differentiated to constitute separate races.

Arthur and Kern[1] also make *Peridermium fusiforme* a synonym of *P. cerebrum*. They state that this conclusion was reached from cultures made by them in 1913; however, their cultural data were not given in this article.

[1] Arthur, J. C., Kern, F. D. North American species of Peridermium on pine. *In* Mycologia, v. 6, no. 3, p. 133–138.

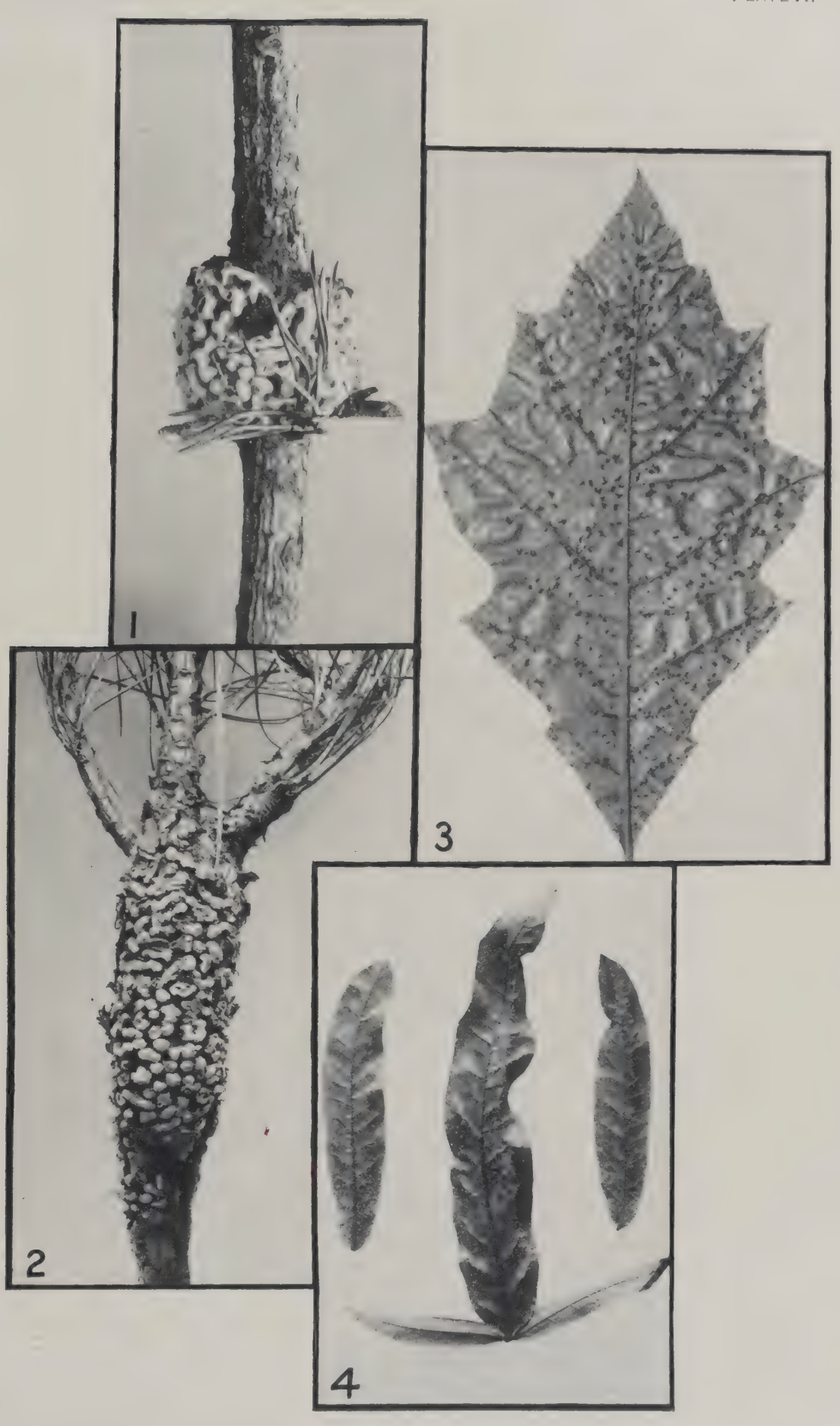
## PLATE XI

Fig. 1.—A globoid swelling formed by *Peridermium cerebrum* on *Pinus echinata*, showing the cerebroid arrangement of the æcia.

Fig. 2.—A fusiform swelling caused by *Peridermium fusiforme* on *Pinus taeda*, showing a similar cerebroid arrangement of the æcia.

Fig. 3.—A leaf of *Quercus rubra* bearing the telia of *Cronartium cerebrum* from an inoculation with æciospores of *Peridermium cerebrum* on *Pinus virginiana*.

Fig. 4.—A leaf of *Quercus phellos* bearing the uredinia of *Cronartium cerebrum* from an inoculation with the æciospores of *Peridermium fusiforme*.


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# IDENTIFICATION OF SPECIES OF FUSARIUM OCCURRING ON THE SWEET POTATO, IPOMOEA BATATAS

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## INTRODUCTION

Students of the etiology of diseases caused by Fusarium are often handicapped by the fact that they get various results from what appears to be the same fungus and obtain like results from apparently different fungi. To throw light on the interpretation of such results and to serve as a guide for future studies, the group of Fusarium presented in this paper may be separated from the remainder of the genus.

Species of Fusarium play an important part in the diseases of *Ipomoea batatas* Poir., the sweet potato. Many of the 13 different species and varieties of this genus of fungi are more or less cosmopolitan and ubiquitous and seem to be harmless to the sweet potato but injurious to other plants. Other species, so far as known, are confined to this host and connected with serious troubles, such as the wilt disease and the dry-rot of the root. Owing to the heavy losses that plant industry suffers from these diseases, a thorough investigation was undertaken, in order to find methods for their control. This investigation, requiring an exhaustive study of the parasitic fungi, was handicapped by the fact that saprophytes are frequently associated with parasites and resemble them in certain stages so closely that they are readily mistaken for them. This led to contradictory reports as to the nature and causes of the diseases. The only way to get uniform results is to base these etiologic studies on a monograph of all the fungi associated with the diseases. This paper, although not exhaustive, includes at least the most important species of Fusarium occurring on the sweet potato. These have been grown in pure culture for almost two years until "criteria of the norm and sub-

norm" could be found and a sufficient constancy of the spores obtained under constant conditions. The diagnoses based exclusively on pure cultures allow also a determination of the fungi from field material when the effect of different degrees of moisture and of various substrata is understood, when what is youth and what old age, and what is mature and what immature in species of Fusarium [1] are known.

## METHOD OF DIFFERENTIATION

The genus Fusarium has a number of vegetative and spore stages,[2] and their so-called variability may be a source of confusion. This is evident from the fact that transfers of mycelium produce a growth quite different in general appearance from that derived from spores of the same fungus to the same medium under conditions otherwise identical. Conidia from the outside and mycelium from the fibrovascular bundles of a wilted plant isolated separately and grown and studied under the same conditions may show differences in general appearance and still be the same organism. They must be regarded as the same organism if one form can be transformed into the other. The following may be cited as proof: Hundreds of wheat grains among samples sent to the Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft, at Dahlem, near Berlin, Germany, to determine the cause of poor germination, showed carmine-red spots of fungous mycelium. Numerous cultures, derived from epiphytic and endophytic mycelium of different seeds, yielded a number of fungi, such as Verticillium, Spicaria, Alternaria, Trichothecium, Langloisula, Ramularia, Melanospora, Leptosphaeria, Helminthosporium, Gibberella, and Fusarium (three species), without an ascus stage.

The four last-named organisms, afterwards found to be distinct, showed practically the same general appearance; as, for example, in a sterile cottony growth of aerial mycelium all four showed yellow on sterilized rice media and carmine red on steamed potato. Repeated transfers of mycelium to the same medium did not give differential characters sufficient to identify any one character by this method. In some cases a few small conidia scattered in the mycelium were developed, but without any constant shape that was characteristic. Within a month, however, some plectenchymatic bodies appeared, often only one or two (in a test-tube culture on potato) pushing through the sterile mycelium. Later, an erubescent to orange color appeared in one culture and an ochreous brown color in another, especially when exposed to daylight. This difference gave the first striking contrast between

[1] Exsiccatæ will be prepared from all of these fungi and, when completed, subcultures of the original strains will be sent to any-one interested.

[2] Such expressions as "mycelial stage," "sclerotial stage," "sporodochial stage," "pionnotes stage," "micro-conidial stage," etc., for the sake of convenience, are used quite generally throughout this article. While they are not to be regarded as true stages in the accepted sense of the word, they are particularly desirable terms to use in connection with a taxonomic study from pure cultures of the genus Fusarium. For definition of terms see Wollenweber, H. W. (1913c).

these species. The spore colors were emphasized through the contrast with the carmine mycelium thallus common to all four strains. These ochreous and orange spots contained masses of characteristic sickle-shaped conidia. One culture was found to have ochreous spore masses of the type illustrated in Plate XVI, fig. *J*, although the majority of cultures contained spores of a longer type (Pl. XVI, fig. *O*). Cultures with orange conidial beds, however, presented a quite different type from both of those just mentioned. This type, although not figured, was more subulate than the forms in Plate XVI, fig. *M*. A second orange type in another culture was of the same general shape, but a little broader. (See Pl. XVI, fig. *G*.) The descendants of single spores of these four types of sickle-shaped conidia, repeatedly transferred to different vegetables, stems, heads, and grains, remained distinct from each other, but constant in themselves in the same spore stage. The sporodochia were formed more freely in subcultures, especially on stems. In addition to this so-called tubercularia-like sporodochial stage, one of the four strains (Pl. XVI, fig. *O*) produced perithecia and was identified as *Gibberella Saubinetii* (Mont.) Sacc. It had no chlamydospores, but thick-walled swollen cells occurred in plectenchymatic bodies (stroma) and in closely interwoven hyphæ (Pl. XIV, fig. *J*), which often resembled chains of chlamydospores, but differed from them in function. The other similar type (Pl. XVI, fig. *J*) never formed perithecia, but produced clusters of chlamydospores. The latter were quite as resistant as perithecia and have proved to be an effective resting stage in species destitute of the ascigerous form. Later, the two other strains of the section Roseum with orange conidia were also found to differ more than was apparent at first glance. The broader type produced blue globose sclerotia in cultures on stems repeatedly watered when dried out. These bodies resembled in general appearance perithecia of Gibberella, but were massed and much smaller, 56 to 70$\mu$ in diameter, and without any indication of asci. Such sclerotia were entirely wanting in the species having the slender type of conidia. The study for some years of these four above-described strains isolated from wheat (*Triticum* spp.) did not change the previous conclusion that they were four separate fungi: *Gibberella Saubinetii* (Mont.) Sacc. (Pl. XIV, and Pl. XVI, fig. *O*); *Fusarium culmorum* (W. G. Sm.) Sacc. (Pl. XVI, fig. *J*); *F. subulatum* App. and Wollenw.; and *F. metachroum* App. and Wollenw. These fungi were formerly included in the collective species *F. roseum* Link autorum.

From this example it is seen that to differentiate these fungi is only a question of method. A skeptic, however, may ask, Why is *Fusarium culmorum* not the conidial stage of Gibberella? It must be conceded that the conidia derived from ascospores of Gibberella resemble in shape those of *F. culmorum* (Pl. XVI, figs. *J* and *O*). It would not be convincing to state that *F. culmorum* has 5-septate conidia with a maximum

length of 45$\mu$ and an average breadth of 7$\mu$, while the Gibberella-Fusarium has 3 to 5 septate conidia, the quinqueseptate ones being up to 60$\mu$ long and 5.5$\mu$ broad, since isolations of both, which have been repeatedly made, from different hosts showed a range of variation in the size of conidia in different strains of the same fungus. For instance, *F. culmorum* from Irish potato (*Solanum tuberosum*) attained only an average diameter of 6$\mu$, and another strain only 6.5$\mu$, instead of 7$\mu$, as in the original strain from Irish potato. Strains from sweet potato and wheat often gave a smaller maximum. On the other hand, strains of Gibberella-Fusarium already studied from wheat, Irish potato seed balls, sweet potato, and stalks of maize reach an average maximum of 6$\mu$, though in general not exceeding 5.5$\mu$. The septation also varies a little in different strains from the same and from different hosts. The facts fully justify skepticism as to a separation of *F. culmorum* and the Gibberella-Fusarium.

However, a constant difference between the *Fusarium culmorum* and the Gibberella series has been discovered. Chlamydospore clusters, which are absolutely wanting in Gibberella, occur in all strains of *F. culmorum*. This means that the ascigerous fungus, having perithecia, has therefore no longer any need of chlamydospores and may have lost this stage. If the formation of perithecia has been prevented, the effused plectenchyma (Pl. XIV, fig. *J*), which also represents the base of the stroma, may function as a secondary overwintering stage. *F. culmorum*, on the contrary, is dependent on chlamydospores if the conidia die from unfavorable conditions. Finally, the conidial stage itself, generally reduced in Gibberella, is highly developed in *F. culmorum*, forming great masses of conidia in sporodochia and in pionnotes. That the conidia of Gibberella are less independent than in the other species is evident from the fact that earlier or later they join by their anastomosing germ tubes and simply serve as a basis for the ascigerous stroma, thus practically losing their existence as a conidial stage. In *F. culmorum*, on the other hand, conidia have been found to be perennial and resistant for years, and, besides, are able to produce chlamydospores from their cells if they are overwatered or exposed to other unfavorable conditions.

Summarizing these results, it is seen that there is no good reason for regarding *Fusarium culmorum*, a representative of the section Discolor, as the conidial stage of Gibberella. Neither can *F. subulatum* and *F. metachroum* be a stage of this ascomycete, since they have conidia characteristic of the section Roseum (Pl. XVI, fig. *G*). This section is often confused with Gibberella, owing to the frequent association of the so-called *F. roseum* Link with Gibberella on grains of cereals, while the true Fusarium of Gibberella is comparatively rare in nature because of its ready metamorphosis into a stroma.

If doubts based only on unproved analogical conclusions suggested by certain relationships in shape and color of single stages are disregarded and the results of pure cultures in comparison with the same organism

grown under natural conditions in the open field are considered, it must be conceded that the taxonomy of these fungi can be based on studies of pure cultures in a normal condition, even when it is impracticable to make comparison with material from nature.

Fungi from nature also contain a great many abnormal stages. It is often very difficult to determine whether the material in hand is normal. As to what is normal or what is different from the rule depends in the end on the personal judgment of the investigator.

Before proceeding to a general discussion of the "Criteria of the norm" it may be stated that the investigations of the problem have shown that species of Fusarium, with and without the perfect form, make a normal growth in artificial media. In other words, pure cultures afford an efficient and convenient basis for taxonomic and pathological studies. Sterilized plant stems give a "mean proportional" of nutrition on which are developed the most characteristic stages without loss of vital power.

## CRITERIA OF THE NORM

A great variety of conditions are found in pure cultures, especially when different media are used, from which it is always necessary to select some and reject others. Although sterilized plant stems gave ordinarily a good growth, the shape, septation, and relative viability of the conidia depended largely on the quantity of water present in the culture, whether grown in light or darkness, whether transferred from agar or gelatin containing strong acids or alkalis, etc. For instance, in *Gibberella Saubinetii* the cells of spores become barrel-shaped from the imbibition of water (Pl. XIV, figs. *H* and *K*, 2) when septate conidia are transferred from a concentrated to a dilute medium.

If, however, the conidia are dried out, a constriction takes place instead, with the result that the septa project ringlike (Pl. XIV, fig. *F*, seventh spore). Under similar influences the ascospores react in the same manner, the cells become swollen (Pl. XIV, fig. *E*, 1), constricted (Pl. XIV, fig. *E*, 3), or remain unchanged (Pl. XIV, fig. *E*, 2). Such reactions, although more striking in the living cells, are not confined to them, but may occur also in killed cells. Vacuoles are often formed in swollen cells, in mature chlamydospores (Pl. XIII, fig. *D*), conidia (Pl. XIV, fig. *H*), in plectenchymata (Pl. XIV, fig. *J*), and in overwatered hyphæ. Vacuoles may occur also in unswollen cells (Pl. XVI, fig. *H*). With some exceptions, such as in chlamydospores and plectenchymata, the production of vacuoles is rarely a characteristic of health and longevity, so that it is of doubtful value for the norm, especially when associated with swellings.

There is a stage, however, which is characterized by neither swollen nor constricted cells that occurs in conidia, ascospores, hyphæ, and more or less in other organs of fungi. From a long experience it may be said that this stage has the highest constancy in average size, curvature,

septation, color, and is further characterized by longevity. Therefore, this stage and all conditions favoring its production may be called the normal stage.

The reliability of the taxonomic work on the Fusarium problem depends, therefore, on the reliability of this criterion of the norm on which the results are based. If we rely on this test, attention must be given to diagnoses based on other criteria of the norm. Many descriptions doubtless include the measurements of swollen and constricted stages of conidia and ascospores and consequently show a much higher range of variation. The average size, of course, gives a smaller range of variation than the absolute size of particular spores. The difficulty is further increased by the fact that the average size can not be based on an average of spores produced on agar, or from a single culture grown on stems, etc., for the reason that constant temperature, light, moisture, and atmospheric humidity can not always be produced. Even though all these environmental factors are constant, variation in the shape and the size of the conidia might result in subcultures by the transfer of a different type of spore. Inconstant environmental and other factors must be expected both in nature and in culture. If the shape, the septation, and the color of the spore are more constant under some conditions than under others, this state may be regarded as normal, the swelling and constriction of spores as abnormal.

An example: One conidium of Gibberella was 9-septate and 100 by 6$\mu$ in size; another which was 9-septate and with swollen cells measured 85 by 8$\mu$, a third which was 2-septate measured 10 by 2.5$\mu$. These would give a range of variation as follows: 2- to 9-septate conidia, 10 to 100 by 2.5 to 8$\mu$. The average of so-called normal spores was 3- to 5-septate, 30 to 60 by 4.25 to 5.5$\mu$.

According to Saccardo (1879, p. 513)[1], the 5-septate conidia measure 24 to 40 by 5$\mu$, while in our Gibberella from wheat grains they average 45 to 60 by 5 to 5.5$\mu$ and have an absolute fluctuation from 38 to 72 by 4.5 to 6$\mu$. This fact shows that if Saccardo's diagnosis is based on the same fungus it includes only comparatively dry conidia. Thus, the results may be comparable if the influence of such factors as moisture is known. In this case comparison was simple because Gibberella is a characteristic fungus and can not be confused with any other. In species of Fusarium without known ascus stages it is more difficult, since it has been proved that most of the species are ubiquitous, or at least are not confined to a special host. In order to identify these species, a large number of strains must be collected from different hosts and compared with the species described from these hosts in the literature, paying careful attention to the conditions under which the species are found and on which the description is based. In many cases this was impossible, and in

[1] Bibliographic citations in parentheses refer to "Literature cited," pp. 284–285.

some the illustration and description gave complete data. The most questionable method, however, was to base identification of a fungus on exsiccatæ alone. Dried specimens often contain stages collected from a moist location, drying out later, causing constrictions and other changes in the general shape and curvature of the spore so that even the collector would not always recognize his own specimens if he did not know the cause of these changes. The cross walls or septa of the spores in overwatered cultures are frequently absorbed, a condition often considered as normal in old genera and many species of Fusarium. It is not known whether species with long sickle-shaped conidia of the unicellular type exist in this genus, but it is evidently not the rule. Unicellular normal spores of the small ellipsoidal stage, however, exist in all species of Fusarium and form a normal stage in some sections, such as Elegans. These unicellular spores aid in the rapid distribution of a disease. In other sections, such as Discolor and Roseum, the unicellular type is normal in particular species, but subnormal in most others. This fact shows that exsiccated specimens of Fusarium containing a mixture of all types of conidia do not allow an exact determination when they are collected from nature.

## I. FUSARIUM Link

### A. SECTION MARTIELLA[1]

[Species in section Martiella are *Fusarium solani* (Mart.) Sacc., *F. martii* App. and Wollenw., *F. coeruleum* (Lib.) Sacc., and *F. radicicola*, n. sp.]

**Fusarium radicicola**, n. sp.

Diagnosis.—Conidia, normally 3-septate, may occur scattered in sporodochia or pionnotes, averaging 30 to 45 by 3.75 to 5$\mu$; 25 per cent of the total number may be 4-septate; 5 per cent may be 5-septate and average 40 to 59 by 4 to 5.25$\mu$. Chlamydospores, 7 to 10$\mu$, agree with those of other species of the section Martiella.

Habitat.—On partly decayed tubers and roots of plants, such as *Solanum tuberosum* in Europe and America (collected by Wollenweber) and *Ipomoea batatas* in the United States of America (collected by Harter and Field).

*Fusarium radicicola* (Pl. XVI, fig. *K*) has the characters of the section Martiella. The conidia are narrower than in *Fusarium solani* (Mart.) Sacc. (sensu strict.), which has 3-septate conidia averaging 30 to 40 by 5 to 6$\mu$ in size and are shorter and have fewer septations than in *F. martii* App. and Wollenw., which has 3- to 4-septate conidia averaging 44 to 60 by 4.75 to 5.50$\mu$ in size. The plectenchymatic mycelium is olive colored on sterilized potato tuber, with all shades from green to brown. The description has been made from a strain isolated from an Irish potato tuber grown in 1912 on the Potomac Flats, near Washington, D. C.

*Fusarium radicicola* resembles slightly the conidial stage of *Hypomyces cancri* (Rutg.), n. comb. (Pl. XIII, fig. *J*), but has no pedicellate base

[1] The author established these sections (Wollenweber, 1913c) with the diagnoses of the species and references to complete previous descriptions (Appel and Wollenweber, 1910).

(Pl. XVI, fig. *K*). It is often isolated from Irish potato, especially from dry tubers affected with stem-end dry-rot. Sometimes it is associated with other organisms, but frequently seems to invade the tuber from the stolon before a cork layer has been formed to protect the stem end from outside infection. It has never produced the perfect stage. So far there is no sound basis for the supposition that it might be a strain of Hypomyces which has lost the power of producing the perfect stage. The fungus occurs in Idaho, Oregon, and California, and probably in other Western States, and also in all of the New England States except the most northern. Its presence on the sweet potato suggests that it might require a higher optimum temperature than its related species, such as *F. solani* and *F. martii*. Inoculation experiments with strains from different sources are desirable in order to throw more light on the relationship of the above-mentioned fungi and their comparative effect on their hosts.[1]

### B. SECTION DISCOLOR

[Species in this section are *Fusarium discolor* App. and Wollenw., *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw., *F. culmorum* (W. G. Sm.) Sacc. (Syn. *F. rubiginosum* App. and Wollenw.), and *F. incarnatum* (Rob.) Sacc.]

**Fusarium incarnatum** (Rob.) Sacc.

*Fusisporium incarnatum* Rob.; Desm., 1849, *in* Ann. Sci. Nat. Bot., s. 3, t. 11, p. 274.
*Fusarium* (*Fusisporium*) *incarnatum* (Desm.) Sacc., 1881, *in* Michelia, v. 2, no. 7, p. 296.
*Fusarium incarnatum* (Desm.) Sacc., 1886, Syll. Fung., v. 4, p. 712.
*Fusarium incarnatum* Desm., ex McAlp., 1899, Fung. Dis. Citr. Trees Austral., p. 106, fig. 141.
*Fusarium neglectum* Jacz. (1912 *in* Bul. Soc. Mycol. France, t. 28, fasc. 4, p. 340–348, fig. 4) is probably a synonym of *F. incarnatum*.

Diagnosis.—As a rule no sporodochia, no pionnotes, and no chlamydospores, but olive-brown plectenchymata of remarkable longevity are produced. The conidia, formed into a salmon-colored powder, are embedded in a floccose lanate mycelium of the same color. The fungus therefore resembles *Fusarium trichothecioides* Wollenw. in general appearance. Subnormal conidia are unicellular or septate, rounded at the ends, seldom pointed (Pl. XIII, fig. *H*, *a*). Normal conidia show characters of the section Discolor (Pl. XVI, *L*), but are less curved and have mostly a conical (Pl. XIII, fig. *H*, *b*), seldom a pedicellate base (Pl. XIII, fig. *H*, *c*). Slender conidia (Pl. XIII, fig. *H*, *b*) occasionally are seen but should not be confused with the section Elegans (compare *F. orthoceras*). Triseptate conidia measuring 20 to 25 by 3.5 to 4.5$\mu$, and 5-septate conidia, 30 to 50 by 3.75 to 5$\mu$, may be predominant; 10-septate conidia occur more rarely. The conidiophores, mostly irregularly branched, show sometimes slightly verticillate ramifications. Chlamydospores are seldom present and are formed intercalated from hyphæ, or occasionally from conidia.

Habitat.—A cosmopolitan species. It is found in the stems, leaves, fruits, roots, and especially the inflorescences. It occurs rarely on monocotyledons, but has been obtained from Zea, Asparagus, and Iris. This species is confined mostly to dicotyledons and has been found on the following genera: Agrostemma, Dianthus, Brassica, Lupinus, Citrus, Ipomoea, Hyoscyamus, Solanum, Rhinanthus, Campanula, Aster, Tagetes, and Tussilago. It occurs in Europe, America, and Australia.

[1] Compare also the notes under *F. orthoceras*, a species connected with jelly end-rot, a serious trouble in California.

Desmazieres gave in 1849 a short and incomplete description of this fungus, calling it *Fusisporium incarnatum* Roberge, following the designation given by Roberge, the collector of the original material, which consisted of affected inflorescences of *Tagetes erecta*. In 1881 the fungus was transferred to Fusarium by Saccardo in Michelia; it also occurs in Sylloge Fungorum (Pls. XIII, fig. *H*, and XVI, fig. *L*). The best description of this cosmopolitan species is given by McAlpine (1899), who well illustrated the conidia from a citrous stem. He found the fungus associated with *Gibberella pulicaris* in October, 1878, at Ardmona, Victoria, Australia.

The present author, although basing this diagnosis on a strain from Ipomoea, has compared pure culture strains from a wide range of hosts. In looking over the flowers in ornamental gardens late in the summer, one can easily see the pink powder formed by this fungus on plants such as Aster, Iris, Dianthus, Campanula, and Tagetes. It occurs on stems and inflorescences and is not confined to ornamental plants, as proved by its presence on the dead stems and seed bolls of Hyoscyamus, a wild plant common in the United States. Determinations of strains from sweet potato showed its occurrence on the roots. In Germany dead stems of Lupinus are also inhabited by *Fusarium incarnatum*. Although the effect of this fungus on the living plant remains to be worked out, its cosmopolitan and ubiquitous nature seems fully established by these comparative morphologic studies.

A relative of *Fusarium incarnatum*, which often has been found associated with Gloeosporium, occurs on the fruit of the banana. This Fusarium, *F. semitectum* B. and Rav., has smaller conidia and in the average fewer septa than *F. incarnatum*. Inoculation experiments are desirable to throw further light on the simultaneous relations of these organisms. This species is different from that described from carnations by Clayton J. Wight (1912), because it develops neither a lemon-yellow nor a wine-red color on rice; it has no true chlamydospores, and the peculiar curvature of the conidia (Pls. XIII, fig. *H*, and XVI, fig. *L*) gives it a special place in the genus.

*Fusarium dianthi* Prill. and Delacr. (Delacroix, 1901) also is not identical with our species. Its relation to diseases of Aster is also doubtful.

*Fusarium incarnatum* is of morphologic interest because of the absence of sporodochia and pionnotes. Such species in general are less constant in form and septation than those with sporodochia, but successive transfers to fresh culture media, such as stems of legumes and tubers of potato, will establish sufficient constancy to justify the interpretation of the illustrated conidia (Pls. XIII, fig. *H*, and XVI, fig. *L*) as normal. *F. incarnatum* may be provisionally placed in the Discolor section until related species now under investigation require a separation, which may be accomplished either by establishing a subsection of Discolor for them

or an independent section which could be called "Lanceolata" because of the conidia being lanceolate, especially when seen from the back (Pl. XIII, fig. *H*, 4).

3. **Fusarium culmorum** (W. G. Sm.) Sacc.

*Fusisporium culmorum* W. G. Sm., 1884, Dis. Field and Gard. Crops, p. 208–210, fig. 92.

*Fusarium Schribauxi* Delacr., 1890, *in* Bul. Soc. Mycol. France, t. 6, fasc. 2, p. 99, pl. 15, fig. 1; Sacc., 1892, Syll. Fung., v. 10, p. 726.

*Fusarium culmorum* (W. J. Sm.) Sacc., 1895, Syll. Fung., v. 11, p. 651.

*Fusarium corallinum* Mattirolo (non Sacc.), 1897, *in* Mem. R. Accad. Sci. Ist. Bologna, s. 5, t. 6, p. 677, fig. 16–17.

*Fusarium rubiginosum* App. and Wollenw., 1910, *in* Arb. Biol. Anst. f. Land- u. Forstw., Bd. 8, Heft 1, p. 108, pl. 1.

Diagnosis.—Conidia scattered in sporodochia or in pionnotes in masses ochreous to salmon colored, 5-septate, averaging 30 to 45 by 5.5 to 7$\mu$, seldom 3 to 4-septate, rarely with a larger or smaller number of septa. The slight constriction at the apical end and the pedicellate base of normal conidia make this fungus a type species of the section Discolor. Conidiophores in sporodochia increase to repeatedly verticillate ramifications with sterigmata and side branches as many as four in whorls. The mycelium thallus has a yellow acid modification (viz, on rice) turning violet with alkaline and a carmine-red basic one (viz, on wheat and potato tuber) turning yellow with acid. Chlamydospores intercalated, single, in chains or in clusters, averaging 7 to 14$\mu$ in diameter.

Habitat.—This species is found in Europe and North America on all parts of partly decayed plants. It is a wound parasite on cereals and causes scab and seedling blight (foot disease). It has been found on the following hosts: Zea, Avena, Triticum, Secale, Hordeum, Lupinus, Gossypium, Ipomoea, Beta, Solanum, Cucumis, Curcurbita, and others.

This diagnosis is based on the original strain from an Irish potato tuber at Dahlem, near Berlin, described as *Fusarium rubiginosum* by Appel and Wollenweber (1910) and is changed only in the minimum average width of conidia and in the distribution of the fungus.

When the writer studied species of Fusarium on the potato in Germany, he isolated *Fusarium culmorum* (Pl. XVI, fig. *J*) from tubers affected with dry-rot and described it as *Fusarium rubiginosum* App. and Wollenw. In pure culture it attracted special attention by its carmine color on steamed potato tuber. This color always indicated its basic modification, while the red turned chrome yellow with acids. An acid-yellow modification of the fungus, which turned violet by addition of alkali, appeared naturally on steamed rice. This fungus was noted by Schaffnit in his important studies of the "Schneeschimmel" of cereals (Schaffnit, 1913); he stated a similar range of color shades and illustrated this organism very well.

The conidia of this fungus are characterized by a thick membrane and very pronounced cross walls (septa). In maturity they are of ochreous color, lighter in small quantities and darker when seen in masses, but in young culture, especially in moist pionnotes on potato tuber, they may have salmon shades. The presence of chlamydospores facilitates the determination when under certain conditions. The conidia

(Pl. XVI, fig. *J*) resemble those of *Gibberella Saubinetii* (Pl. XVI, fig. *O*). The latter ascomycete has no chlamydospores, as pointed out by the writer (Wollenweber, 1913c, p. 31). This fungus did little damage on potato tuber in several inoculation experiments. Its more destructive nature as a cause of damping-off of oats and wheat (Wollenweber, 1913c, p. 31, 45) has been established by E. C. Johnson (1914). L. H. Pammel (1905) and others have repeatedly called attention to *Fusarium culmorum* as a cause of blight of wheat, barley, and oats in America. Schaffnit (1913, p. 612) described similar effects of the fungus on artificially weakened seedlings of rye.

We can not overlook the fact that students of the "Schneeschimmel" and foot disease of cereals are still endeavoring to appoint perfect stages for species of Fusarium. Voges (1913) illustrates two species of Fusarium on cereals which we easily recognize as *F. metachroum* App. and Wollenw. and *F. culmorum* and thinks he has proved the latter to be the conidial stage of *Ophiobolus herpotrichus* Fr. (Voges, 1912). The present writer, however, has studied Ophiobolus in pure culture and grew normal perithecia on steamed stems of Lupinus and on straw. No sickle-shaped conidia developed in pure culture from any of the stages of the fungus, and no Ophiobolus appeared in pure cultures of any of the species of Fusarium associated in nature with this ascomycete. Voges's studies do not seem to be based on sufficient successful pure-culture work to withstand an unfavorable criticism of his conclusions.

The writer was especially interested in the geographic distribution of *Fusarium rubiginosum*, a study begun in Dahlem. He mentioned (1911, p. 21) its occurrence on Zea, Triticum, and Avena. In the United States this fungus was sometimes isolated from Ipomoea, but its predominant presence on many cereals seems to indicate its better adaptation to the Gramineae, especially to those grown in the warmer part of the Temperate Zone. These facts made desirable a second revision of the described species of Fusarium on cereals, as a result of which it was found that the name *F. rubiginosum* should give way to *F. culmorum*. The illustration (Smith, 1884, fig. 92) leaves no doubt of its identity with this species. It has also been found in France, where Delacroix called it *F. Schribauxii*. Mattirolo (1897), in his interesting studies on Cerebella, seems to have worked with the same fungus. He calls it *F. corallinum* Sacc., but illustrates swollen conidia of *F. culmorum*.

It may be noted that the name "Cerebella" refers to an old genus established by Cesati in 1851. Among the various fungi connected with this stage, *Fusarium* spp. play a part, and Mattirolo's studies suggest that it is the pionnotes stage developed by some species which has caused the confusion with Cerebella.

C. SECTION GIBBOSUM

[Species in section Gibbosum are *Fusarium gibbosum* App. and Wollenw., *F. falcatum* App. and Wollenw., *F. sclerotium* Wollenw., *F. caudatum*, n. sp., *F. caudatum*, var. *volutum*, n. var.]

4. **Fusarium caudatum**, n. sp.

Diagnosis.—Conidia with a tail or whiplike prolongated apical cell and a pedicellate base with well-marked heel, ochreous to salmon colored in mass, formed in sporodochia and in pionnotes; 5-septate conidia averaging 40 to 80 by 3 to 4.5$\mu$, lower and higher septations more rarely occur. Brown chlamydospores, 7 to 14$\mu$ in diameter, as a rule intercalated in chains or clusters, but frequently single if formed from the content of the cells of conidia under poor conditions, such as in water.

Habitat.—On partly decayed stored sweet potatoes (*Ipomoea batatas*) from Clemson College, S. C. (Collected by Harter and Field.)

This species differs mainly from *Fusarium gibbosum* in having more slender conidia, with a prolonged apical cell and a less pronounced hyperbolic curve of the dorsal side.

The hyperbolic curvature of the conidia, when seen in side view, is not as pronounced with *Fusarium caudatum* (Pl. XVI, fig. *M*) as it is with *F. gibbosum*. This is due to the more slender form of *F. caudatum*. All the other characters point at once to the section Gibbosum. The long and whiplike projection of the apical cell, the pedicellate base, with the long foot adorned by a heel (Pl. XVI, fig. *M*), the clusters and chains of chlamydospores, the formation of intra- and extra-cellular conidio-chlamydospores (see Pl. XVI, fig. *A*, 4) in very moist culture conditions—all these are invariably connected with species of this section, such as *F. gibbosum*, *F. sclerotium*, and *F. falcatum*. The morphology of the first species is referred to; this is completely described by Appel and Wollenweber (1910). *F. caudatum* differs in having a more slender form, being longer and narrower when compared with *F. gibbosum* (30 to 60 by 4 to 5.25$\mu$). The tail or whiplike prolonged apical cell is a special character of this fungus, which also forms an abundant pionnotes when single 5-septate conidia are transferred to steamed potato tuber.

A similar but higher septate species of the same section occurs on potato stems, but has more septa and conidia up to 100$\mu$ in length. This and a strain from pine seedlings require more study before a definite determination can be made.

However, a variety of *Fusarium caudatum* with more curved and smaller conidia is added here and named *F. caudatum*, var. *volutum* (Pl. XVI, fig. *P*). Both forms are isolated only from sweet potatoes. Their frequency and distribution remain uncertain.

Comparative studies on chlamydospores show that the section Gibbosum deserves a higher rank in the genus than the section Discolor, although both show a remarkable development in the production of this hibernating form. Clusters and chains of chlamydospores are produced in species of both sections. They may even predominate if water has been liberally furnished. The simplest form of a chlamydospore is a globose or pyriform cell with a thick membrane consisting of at least two

layers in maturity. Two-celled chlamydospores, often produced among unicellular ones, are the first indication of a higher development. Irregular, formless clusters consisting of many chlamydospores are still higher in rank, and the highest type seems to be that cluster distinguished by a true spherical form. Such spheres have been produced only in *Fusarium sclerotium* Wollenw. of the section Gibbosum (Wollenweber, 1913c, p. 32), and all intermediate stages from the unicellular spore to this blue solid body could be observed in pure cultures on steamed wheat heads and potato tubers. These bodies are doubtless true sclerotia. Their peripheric layer has large cells of dark-blue color. The central part is almost colorless and of small-celled parenchymatic structure when sclerotia 50 to 80$\mu$ in size are studied in cross section.

The fact that these sclerotia can be traced back to unicellular chlamydospores proves the close relation of these two resting stages, and the unsuccessful attempt to produce a perfect form in this species leads to the opinion that sclerotia replace the perfect stage in this species of Fusarium. There is no basis for the conclusion that these massive bodies might represent immature perithecia, because Gibberella, Nectria, and Calonectria never have such a uniform structure in the center of the perithecia in similar phases of their development. Consequently it may be concluded that the section Gibbosum has species with chlamydospores, such as *F. gibbosum*, *F. falcatum*, and *F. caudatum*, and other species with sclerotia besides chlamydospores, such as *F. sclerotium*, but none with a known perfect stage. While the section Discolor has species with chlamydospores, it has none with sclerotia and none with a known perfect stage.

5. **Fusarium caudatum,** var. **volutum,** n. var. (Pl. XVI, fig. *P*).

Diagnosis.—Conidia 3- to 5-septate, averaging 25 to 50 by 2.5 to 4$\mu$ in size. Conidia more curved, smaller, and with fewer septa than *Fusarium caudatum*, but agreeing in all other characters.

Habitat.—On partly decayed stored sweet potatoes (*Ipomoea batatas*), La Fayette, Ind. (Collected by Harter and Field).

### D. SECTION ELEGANS

[Species in this section are *F. oxysporum* Schlecht., *F. hyperoxysporum*, n. sp.; *F. tracheiphilum* Smith; *F. vasinfectum* Atk.; *F. lycopersici* Sacc.; *F. niveum* Smith; *F. redolens* Wollenw.; *F. orthoceras* App. and Wollenw.; *F. orthoceras*, var. *triseptatum*, n. var.; *F. batatatis*, n. sp.; and *F. conglutinans* Wollenw.]

6. **Fusarium orthoceras** App. and Wollenw.

*Fusarium orthoceras* App. and Wollenw., 1910, *in* Arb. Biol. Anst. f. Land- u. Forstw., Bd. 8, Heft 1, p. 1–207, 10 fig., 3 pl.

*Fusarium orthoceras* App. and Wollenw., Wollenweber, 1913, *in* Phytopathology, v. 3, no. 1, p. 30.

Diagnosis.—Conidia as a rule unicellular, averaging 5 to 12 by 2.5 to 3.5$\mu$, embedded in a cottony mycelium layer, often jellied with age. No sporodochia, no pionnotes, and no sclerotia. A few, not exceeding 15 per cent, of the conidia, averaging 25 to 46 by 3 to 4$\mu$, may be 3-septate. Septal zone nearly cylindrical, slightly curved at the apical end, which is unequilateral-conical; the base is nearly straight-conical and may or may not end in a very reduced foot; 4- and 5-septate conidia rare, averaging up to 50 by 4.0$\mu$; conidiophore with irregularly arranged sterigmata, seldom tri-

furcate. The fungus is ochreous to salmon colored in light, in darkness it fades to brownish white. Thalloplectenchymata wine red in the acid modification (on rice) and blue spotted in the basic modification (on potato tubers); but sclerotial plectenchymata entirely wanting. Chlamydospores intercalated, globose to ovoid, 1-celled forms averaging 7 to 10$\mu$, 2-celled forms more rare but with a somewhat larger major axis.

Habitat.—A cosmopolitan species. Inhabits the root system of such plants as Allium, Brassica, Ipomoea, Solanum, Capsicum, Apium, Citrullus. This species is especially abundant in North America. Probable cause of jelly end-rot of potato tubers and root troubles.

In Europe this fungus is confined to the cooler part of the Temperate Zone. In Germany and Norway it is common on the tuber, the stolon, and other parts of the root system of *Solanum tuberosum*. In America at least 20 strains of this fungus from potato have been added to the writer's collections, which were mostly transferred to him in pure culture for determination. The following States may be recorded as sources of this material: Tennessee, New York, Vermont, Maine, Ohio, Illinois, Iowa, Wisconsin, Minnesota, Washington, Oregon, California, and the District of Columbia. Furthermore, *Fusarium orthoceras* was sent with potatoes from Cape Colony, Africa, and from Chiloe, South America, also from Great Britain. These additional isolations proved its cosmopolitan nature. In the Bureau of Plant Industry, Washington, D. C., the fungus was frequently isolated and determined from diseased plants, such as onion, cabbage, sweet potato, celery, and occasionally from watermelon (Citrullus) and sweet pepper (Capsicum). The determination of this species is somewhat handicapped by the predomination of unicellular conidia, but repeated transfers and variation of media are sufficient means to provide sickle-shaped septate spores of typical form. The conidia and the chlamydospores, the mycelium with its jellied growth in old cultures, and its deep wine-red to purple-acid modification (turning blue with alkali) on rice allowed the final determinations

Only a few, not exceeding 15 per cent, of the conidia are 3-septate, as indicated in the diagnosis of the type strain studied in Berlin. This low percentage of the characteristic sickle-shaped conidia was quite uniformly found in many strains from the same or from different host plants. However, one strain out of several isolated from *Ipomoea batatas* had as many as 100 per cent of 3-septate conidia (Pl. XVI, fig. *N*) under the same conditions, where the other strains had but a few per cent of them.

7. **Fusarium orthoceras,** var. **triseptatum,** n. var.

Diagnosis.—Differs from *Fusarium orthoceras* in having a higher septation of conidia, by the presence of sporodochia, and a reduced pionnotes. Under normal conditions as many as 100 per cent of the conidia are 3-septate. Ten per cent of 4- and 5-septate conidia are found. Unicellular conidia and chlamydospores occur and prevail under certain conditions.

Habitat.—On partly decayed roots of *Ipomoea batatas*, Newark, Del. (collected by Taubenhaus, 1912).

In order to find whether or not one type strain of *Fusarium orthoceras*, var. *triseptatum* (Pl. XVI, fig. *N*), could be split up into strains with different percentages of unicellular and septate conidia, the writer separated 20 conidia from a subculture of a single spore and transferred each to a test tube on potato tuber. Some of these conidia were sickle-shaped and septate, but most of them unicellular. No differences could be observed as to a remarkable increase of 3-septate conidia, nor was there a sign of sporodochia. While the exceptional strain exposed to the same treatment did not degenerate to a strain with predominantly unicellular conidia, it produced sporodochia on stems in contrast to the type strain. It seems better, therefore, to separate this strain provisionally from the others as a variety, unless this contradiction is cleared up by more advanced culture methods. The name "*F. orthoceras*, var. *triseptatum*, n. var.," is therefore proposed.

*Fusarium orthoceras* and its variety are provisionally included in the section Elegans, although their conidia have no true bottle-shaped apical cell and scarcely a pedicellate base. Inoculation experiments are needed to ascertain whether or not this fungus is the cause of one type of jelly end-rot of potato tubers. In Watsonville, Cal., in October, 1913, the writer found up to 80 per cent of Burbank potatoes in a large acreage affected by this peculiar soft rot, which is quite different from that produced by *F. coeruleum* and other species. Every year specimens with this disease are sent from California, especially from the moorland (called "tule district") of the San Joaquin Valley. In tubers with the jelly end-rot *F. orthoceras* is often, but not always, associated with such fungi as *F. radicicola*, *Mycosphaerella solani*, *Sporotrichhum flavissimum* Lk., Rhizoctonia, and also with bacteria. It may be repeated (Wollenweber, 1913c, p. 30) that *F. orthoceras* has been sent to the writer from various sources in pure culture under the name "*F. oxysporum* Schlecht." and that *F. gibbosum* has been sent under the name of the latter species. The complete descriptions and illustrations of these fungi will help to determine and differentiate them, and future inoculation experiments, based on these well-known organisms, will succeed in reducing contradictory reports to a minimum.

The section Elegans plays the most important part in the species of Fusarium on sweet potato because it comprises 4 species determined out of 16 strains from various sources, isolated and reisolated from Ipomoea. When all of them were at the height of growth, the morphologic characters were noted and illustrated. Then inoculation experiments, carried out by L. L. Harter and Ethel C. Field, showed that strains reisolated from successfully inoculated plants remained as constant as in the old cultures. This has been proved to be so, and the considerable time devoted to get these results seems to have been well spent. Two years ago, when this study began, it was the opinion of the

writer that all strains belonging to the section Elegans agreed closely enough to justify their determination as varieties of *Fusarium vasinfectum* Atkinson. At the height of growth, however, the conidia differed in the finer form, the size, septation, and in color shades. The production of sporodochia and pionnotes, the color of mycelium, and such functions as the pathogenicity offered other determinative factors. Secondary characters, such as the production of odors on steamed rice, wheat, and corn, also aided the differentiaton. Two species, their subcultures and reisolations, are proved to be xylem parasites by Harter and Field. These species produced wilt disease, while others refused to do so. The latter agreed in morphology either with *F. oxysporum* or with *F. orthoceras*, the former differed and have been described as *F. batatatis* and *F. hyperoxysporum*. The differences illustrated in Plate XVI, figs. *D* and *F*, and described in the diagnosis are striking to the writer, but he knows that there is one weak point which allows criticism of his opinion. He was unsuccessful for two years in the attempt to transform one of these fungi into the other. However, it is of fundamental value and of vital importance in the classification to find out whether these conclusions are right or wrong, and strains of the various fungi of this section will be available to anyone who may care to throw further light on the complicated problem of drawing the border line between species, subspecies, varieties, and subvarieties, or proving that border lines do not exist to the extent claimed in this paper.

It seems at first probable that there is only one fungus causing the same disease on one host, but okra (*Hibiscus esculentus*) and eggplant (*Solanum melongena*) are attacked by Verticillium in one district and by Fusarium in another district, although the wilt symptoms are so similar that isolation and study of the parasite are the only means of deciding which fungous genus is connected with the trouble in each case. Furthermore, at least three serious wound parasitic species of Fusarium cause potato tuber-rot. Here, also, the isolation of the fungus, not the general appearance of the tuber, decides the question which parasite does the damage. In some cases the climate and methods of cultivation are essential. They offer ideal conditions for one fungus, but not for the other. But this is not always so, and the zone of distribution of one fungus overlaps that of the others. These facts may become important for the control of disease, and the good results performed by an exchange of seed may not be due simply to climatic factors, but to the weakening of parasites by a change of their optimum environmental conditions. Of course, a change of climate may be fatal to the fungus and its host, but there is no reason why it should not be supposed that a zone exists where the host thrives well and the parasite degenerates. Often, however, if one parasite has been successfully controlled, there will soon be another. Control methods may be greatly aided by a careful study of the parasites. In finding what is

normal, abnormalities and conditions which lead to them may be determined. Abnormalities of one fungus, unfortunately, may resemble the norm of another. Without studying the criteria of the norm, two fungi may be mistaken for one. Literature shows that this error leads to more trouble than the mistake of drawing too sharp a border line between closely related species.

*Fusarium batatatis* differs from *F. hyperoxysporum*. The conidia of the former are 11 to 13 times longer than broad (Pl. XVI, fig. *D*), while those of the latter are 8 to 9 times longer than broad (Pl. XVI, fig. *F*). The apical cell is slender in the former, while bottle-shaped in the latter. The base of the latter is more pronouncedly pedicellate than in *F. batatatis*. The microconidial stage prevails in *F. batatatis*, giving the pure culture on steamed potato tuber a powdery appearance. However, a few of the larger septate sickle-shaped conidia will be found scattered among microconidia. These macroconidia (Pl. XVI, fig. *D*), repeatedly selected, and cultured separately, will result in the reduction of the microconidia and the aerial mycelium to a minimum, so that a perfect pionnotes (Pl. XII, fig. *B*) can be produced on the very same substratum. This pionnotes produces abundant chlamydospores (Pl. XVI, fig. *B*) and redevelops microconidia in old age (Pl. XVI, fig. *C*), which look like a parasitic growth on the spore slime of the former. There is no difficulty in favoring the production of a particular stage, and one stage can easily be transformed into the other, but the constant tendency to form microconidia is characteristic of this fungus. In order to study the stroma, transfers of mycelium or microconidia were made to potato cylinders. The fungus rapidly formed a thallus-like layer (stroma) covering the surface of the substratum. When the potato was sufficiently decomposed and its water consumed by the fungus, the stroma became shriveled. Blue blisters of sclerotial structure smaller than a pinhead appeared at this time by hundreds within the stromatic layer. These sclerotial bodies (Pl. XII, fig. *A*) correspond to the stroma of sporodochia as proved in parallel cultures on steamed stems of sweet potato, potato, or sweet clover. They develop within the epidermis of the stems and often push through in order to develop a convex layer of the sickle-shaped macroconidia. The stromatic thallus on potato tuber is unnecessary for the fungus on stems, and is replaced by endodermatic hyphæ which develop the sclerotial base of the sporodochia described. The blue bodies having a plectenchymatic structure may be called sclerotial plectenchymata. If they are spherical, as in *F. sclerotium*, the writer has called them sclerotia, and there is reason enough for not drawing too sharp a distinction between these different types.

The blue color turns red with acids, and a wine color on steamed rice (Pl. XII, fig. *C*) which turns blue with alkali shows the reverse effect of the reaction the fungi produce when grown on different media.

*Fusarium hyperoxysporum* has larger but fewer sclerotial bodies on potato tuber. Consequently the sporodochia are larger in this species than in *F. batatatis*. A lilac odor is present in cultures on rice, also on potato tuber, but weaker, while *F. batatatis* has a weak alcoholic odor. A perfect pionnotes can be easily produced with *F. hyperoxysporum* on potato tuber, but is always accompanied by or embedded in aerial mycelium. This fungus resembles more closely *F. oxysporum*, which, however, does not produce wilt disease on Ipomoea. While *F. batatatis* is related to *F. orthoceras*, it differs in characters of the sporodochia, which may be compared in the diagnosis. A number of finer details can be found.

**8. Fusarium batatatis**, n. sp. (Pls. XII and XVI, figs. *A–E*).

Diagnosis.—Conidia both scattered and in sporodochia or pionnotes, when scattered mostly unicellular. Conidia mostly 3-septate, rarely 4- and 5-septate, when in sporodochia or pionnotes ochreous to salmon colored. Brown chlamydospores and blue sclerotial plectenchymata present. Conidia measure as follows: Unicellular forms, 5 to 12 by 2 to 3.5$\mu$; 3-septate, 25 to 45 by 2.75 to 4$\mu$; 4- to 5-septate, 37 to 50 by 3 to 4$\mu$. Brown chlamydospores, 7 to 10$\mu$ thick and similar to those of *F. orthoceras*. The septate conidia are of the same size as corresponding conidia of *F. orthoceras* and differ in shape from *F. oxysporum* in being a little more slender. The blue sterile sclerotial bodies at the base of the sporodochia have a blister-like appearance between the felty powdery dry stroma of scattered conidia and push either through the epidermis or stems or the thallus covering the substratum. According to recent investigations of L. L. Harter and Ethel C. Field, this species causes the wilt (stem-rot) of *Ipomoea batatas* by invasion of the fibrovascular bundles of the stems and roots. (Collected by Harter and Field.)

**9. Fusarium oxysporum** Schlecht.

*Fusarium oxysporum* Schlecht., 1824, Fl. Berol., pars 2, p. 139.
*Fusarium oxysporum* Schlecht., Erw. Sm. and D. B. Swing., 1904, *in* U. S. Dept. Agr. Bur. Plant Indus. Bul. 55, 64 p., 8 pl.
*Fusarium oxysporum* Schlecht., Manns, 1911, *in* Ohio Agr. Exp. Sta. Bul. 229, p. 299–336, illus.
*Fusarium oxysporum* Schlecht., Wollenw., 1913, *in* Phytopathology, v. 3, no. 1, p. 28, 40–45, illus.

Diagnosis.—Conidia both scattered and in sporodochia or reduced pionnotes, in mass ochreous to salmon colored. Unicellular conidia, 5 to 12 by 2 to 3.5$\mu$; 3-septate, rarely 4- and 5-septate, conidia from sporodochia, 25 to 42 by 3.25 to 4.75$\mu$. Sclerotial plectenchymata blue with rough surface or even sphærostilbe-like projections. Brown chlamydospores, 7 to 10$\mu$ in diameter. This species has a slight lilac odor on steamed rice and milk.

Habitat.—A vascular parasite, cause of wilt disease, but not tuber-rot, of *Solanum tuberosum* in the United States of America, districts of South America and Australia. Also found on various hosts, such as Lycopersicum, Vigna, Pisum, and Ipomoea.

**10. Fusarium hyperoxysporum**, n. sp. (Pl. XVI, *F*).

Diagnosis.—In morphology of various spore forms and the slight lilac odor, this species agrees with *F. oxysporum*, but differs in having a perfect pionnotes. This species, however, differs biologically from *F. oxysporum*, since it has been proved by L. L. Harter and Ethel C. Field to cause a wilt disease (stem-rot) of *Ipomoea batatas*, but not of *Solanum tuberosum*. On the other hand, *F. oxysporum* causes the well-known wilt disease of *Solanum tuberosum*, but is not infectious on *Ipomoea batatas*.

E. SECTION ROSEUM

[Species of this section are *F. subulatum* App. and Wollenw., *F. metachroum* App. and Wollenw.; *F. putrefaciens* Ostw.; and *F. acuminatum* Ell. and Ev.]

11. **Fusarium acuminatum** Ell. and Ev.

*Fusarium acuminatum* Ell. and Ev., 1896, *in* Proc. Acad. Nat. Sci. Phila., 1895, p. 441.
*Fusarium acuminatum* Ell. and Ev., Sacc., 1899, Syll. Fung., v. 14, p. 1125–1126.

Diagnosis.—Conidia, scattered, in sporodochia or in pionnotes, orange in mass. Conidia average as follows: 5-septate, 40 to 70 by 3 to 4.5$\mu$; 4-septate (less common), 30 to 60 by 3 to 4.5$\mu$; 3-septate, 20 to 45 by 2.75 to 4.25$\mu$. Conidia of 0-, 1-, 2-, 6- and 7-septations are occasionally found. Subnormal small conidia may be mistaken for conidia of the section Discolor, but normal sporodochia develop on repeatedly whorl-like branched conidiophores, giving the characteristic conidia of the section Roseum. The conidia show in side view hyperbolic or parabolic curves, in contrast to *Fusarium metachroum* App. and Wollenw., the conidia of which are as a rule more nearly straight. Blue globose sclerotia, 50 to 70$\mu$ thick, occur and form a striking contrast to the carmine plectenchymatic thallus on starchy media, such as steamed potato tubers. Both blue and carmine are basic modifications of the fungus, while yellow (on rice) is the acid one, turning blue to purple violet with the addition of an alkali.

Habitat.—Occurs on partly decayed plants, especially on stems, roots, and tubers, also on fruits. Found on Solanum, Ipomoea, Fagus (beech nuts), and *Impatiens balsamina* in the United States of America.

Conidia of *Fusarium acuminatum* (Pl. XVI, fig. *G*) have the parabolic dorsal and ventral curvature which is characteristic of the section Gibbosum, but less pronounced in the section Roseum. Gibbosum, however, requires the presence of chlamydospores and the absence of carmine mycelium, while this fungus has no chlamydospores but carmine mycelium. Therefore, it has to be classed under the section Roseum. The mycelium becomes yellow on steamed rice. This acid yellow modification turns violet with alkali. This fungus is more distributed on Solanum than on Ipomoea and has also been found on Impatiens and on beech nuts (Fagus) in the United States. Its conidia (Pl. XVI, fig. *G*) are more curved and more swollen towards the middle of the septal zone than *F. metachroum*, but a remarkable relationship to the latter can not be overlooked.

The strains from Solanum and from Ipomoea agreed in all respects. The diagnosis is derived from a strain isolated from a potato tuber, but many isolations of the same fungus are made from potato stems. The strains from beech nuts and Impatiens were a little more slender and had a whiplike prolonged top cell often resembling that of *Fusarium caudatum*.

Ellis and Everhart (1896) described *Fusarium acuminatum* as follows:

Sporodochia gregarious, minute, white at first, then flesh-colored. Conidia falcate, attenuate-acuminate at each end, 3–5, exceptionally 6 septate, not constricted, arising from slightly elongated cells of the proligerous layer, in which respect it differs from the usual type of Fusarium. . . .

Saccardo's translation (1899) of this diagnosis in Sylloge Fungorum is:

Sporodochiis gregariis, minutis, ex albo carneis; conidiis falcatis, utrinque attenuato-acuminatis, 3–5–, rarius 6 septatis, ad septa non constrictis, e cellulis subelongatis oriundis. *Hab.* in caulibus vivis Solani tuberosi, New York, in America boreali.

This description is incomplete, like many others, but the writer found this fungus so widely distributed on potato stems in the New England States that he feels justified in identifying it as *Fusarium acuminatum*. Blue sclerotial plectenchymata appear in pure cultures on stems of Melilotus, Zea, and a blue color occasionally develops in spots on plectenchymatic bodies on potato tuber and wheat grains.

## II. HYPOMYCES (Fr.) Tul.

12. **Hypomyces ipomoeae** (Hals.) Wollenw. (Pls. XIII, figs. *A–G;* XV, fig. *A*, *1–6;* and XVI, fig. *H*).

*Nectria ipomoeae*, Hals., 1892, *in* N. J. Agr. Exp. Sta., 12th Ann. Rpt., 1891, p. 281–283, fig. 20–22.
*Creonectria ipomoeae*, Seaver, 1910, *in* N. Amer. Fl., v. 3, pt. 1, p. 22.
*Nectria coffeicola*, Zimm., 1901, *in* Centbl. Bakt. [etc.], Abt. 2, Bd. 7, No. 3, p. 103–106, fig. 6.
*Hypomyces ipomoeae* (Hals.) Wollenw., 1913, *in* Phytopathology, v. 3, no. 1, p. 34.
*Nectria saccharina*, Berk. and Curt., 1869, *in* Jour. Linn. Soc. [London], Bot., v. 10, p. 378, no. 766, is probably a synonym of *H. ipomoeae*.
*Nectria Goroschankiniana*, Wahrl., 1886, *in* Bot. Ztg., Jahrg. 44, No. 29, p. 503, pl. 3, fig. 17, 22, 25, is probably a synonym of *H. ipomoeae*.

Diagnosis.—Perithecial stage: Perithecia scattered or gregarious, free on the surface of the host as well as embedded in mycelium or on a distinct plectenchymatic stroma, ovoid, subconical, subflask-shaped, averaging 225 to 375 by 175 to 300$\mu$. Peridium strongly verrucose, owing to protuberance-like projections of cell groups, red to reddish brown, except the almost colorless conical beak. A few paraphyses line the inner wall of the throat from the ascus ball to the ostiolum. Asci up to over a hundred in each perithecium, intermixed with a few more celluled paraphyses. Ascospores, 8 in one row or irregularly in two rows, 2-celled ovoid to ellipsoidal with wrinkled exospore, in mass brownish white; one septum, average size, 10 to 13 by 4.5 to 6$\mu$, undermoist overripe condition slightly constricted at the septum. Conidial stage: Conidia scattered in sporodochia or pionnotes, of nearly cylindrical shape at the septal zone, slightly pointed and curved at the ends, base pedicellate without a distinct heel. Conidia, 3 to 5 septate; 3-septate, 30 to 45 by 3.75 to 5$\mu$; 5-septate, 45 to 70 by 4.25 to 5.5$\mu$. Of the total number, 30 per cent may be 6-septate, 10 per cent may be 7-septate, with an average size up to 70 by 6$\mu$. In young, moist, and hunger stages unicellular conidia occur, averaging 6 to 12 by 3 to 4.75$\mu$. Color of conidia masses brownish white, occasionally impregnated with blue, a mycelium color, especially formed in the plectenchymata. Conidiophores verticillately branched. Chlamydospores globose or ellipsoidal, terminal and intercalated, mostly unicellular and scattered, average diameter, 7 to 10$\mu$.

This diagnosis is derived from subcultures of a strain isolated by Dr. Donald Reddick, of Cornell University Agricultural Experiment Station, from a badly rotted sweet potato sent him on April 30, 1907, by J. M. Van Hook, of the Ohio State Agricultural Experiment Station, Wooster, Ohio.

Habitat.—Saprophyte on dead parts of plants. Probably a cosmopolitan and ubiquitous species. Found in North America on *Solanum melongena*, New Jersey; *Ipomoea batatas*, Clemson College, S. C.; and Vineland, N. J. (L. L. Harter and Ethel C. Field, 1912); Newark, Del. (J. J. Taubenhaus, 1912). Also in Asia on *Coffea arabica*, *Melia azedarach*, *Theobroma cacao* (fruit), Java (Zimmermann, sub *Nectria coffeicola*, 1901); *Glycine hispida* Max., *Phaseolus mungo*, var. *subtrilobata* Province Higo, Japan (Yoshino, 1905). In Africa on Cinchona (Zimmermann, sub *Nectria coffeicola*, 1904). In Europe on *Orchydeae* (roots; Wahrlich, sub *Nectria Goroschankiniana*, 1886).

12a. **Hypomyces cancri** (Rutgers), n. comb.[1] (Pls. XIII, *J*, and XV, *B–C*).

*Nectria cancri* Rutg., 1913, *in* Ann. Jard. Bot. Buitenzorg, v. 27 (s. 2, v. 12), pt. 1, p. 62.

Diagnosis.—In general appearance this species resembles *Hypomyces ipomoeae*, but differs in having a lower average septation of the conidia, larger perithecia, and larger ascospores. Contrary to *H. ipomoeae*, the conidial stage, especially sporodochia and pionnotes, is more prevalent than the ascigerous stage. Perithecia average 350–450 by 275 to 375$\mu$; ascospores average 10 to 15 by 5 to 6.75$\mu$ in size; conidia largely 3-septate average 30 to 45 by 3.75 to 5$\mu$ (3 to 5 septate conidia average 30 to 55 by 3.75 to 5.5$\mu$ in size).

Habitat.—On cankered bark of *Theobroma cacao* in Java (Rutgers), on dead taproots of *Cannabis sativa*, Potomac Flats, Washington, D. C., North America (Wollenw.). This diagnosis is based on pure cultures of the strain isolated from Cannabis.

*Hypomyces ipomoeae*, well known as *Nectria ipomoeae* Halsted (1892), has both true chlamydospores (Pl. XIII, fig. *D*) and conidia of the shape described for similar Hypomyces, such as *H. solani* Reinke and Berthold (1879). Such chlamydospores are lacking in pure cultures of Nectria, as proved by the writer for the sections Willkommiotes and Tuberculariastrum (Wollenweber, 1913b, p. 203–204, 226–229). A transfer of *N. ipomoeae* to the genus Hypomyces is therefore advocated (Wollenweber, 1913c, p. 34). Since further taxonomic discussion on this fungus will be aided by more complete descriptions, additional notes and illustrations (Pls. XIII and XV) have been given here.

This saprophyte, now known to be cosmopolitan and ubiquitous, has crossed the path of various pathologists who always thought it was a new species when they isolated it from other hosts than eggplant and sweet potato. So long as the opinion of its parasitic nature prevailed, an adaptation to particular hosts could be supposed, but its saprophytic nature established by Harter and Field for sweet potatoes and eggplants and by the writer for eggplant leaves no doubt of its indifference regarding the host.

In pure culture the easy and rapid development of perithecia on almost any steamed vegetable recalls the similar omnivorous conduct of other saprophytes, such as Melanospora and Chaetomium, while *Gibberella Saubinetii* and *Nectria discophora* favor some media more than others for the production of the perfect form, and *Nectria galligena* and *Calonectria graminicola* require a careful selection of media for the completion of their life cycle in pure culture.

*Hypomyces ipomoeae* has been studied in a number of strains from Ipomoea, sent from Ohio, Delaware, and New Jersey, and isolated by L. L. Harter and Ethel C. Field, Bureau of Plant Industry; J. J. Taubenhaus, Delaware Experiment Station; and Dr. Donald Reddick, Cornell Experiment Station. No essential differences could be detected within two years, and it should be noted that Reddick's strain has been carried through cultures since 1907 without showing any degeneration or any

[1] This fungus from hemp, although not yet found on sweet potato, has been discussed in connection with *Hypomyces ipomoeae*, and it was thought advisable to give a short description.

change in its known characters. Sometimes the production of conidia preceding that of the perfect form is more abundant and lasts longer in one culture set than in another of the same strain, but the constancy has been found a function of the constancy of method and medium. The conidia showed a remarkable constancy in shape and size (Pl. XIII, fig. *G*) and had 3 to 5 septa. There was, of course, a fluctuation in the percentage of 3-, 4-, and 5-septate conidia, depending on the medium itself and on the age of the culture. Once the triseptate conidia prevailed, and sometimes the quinqueseptate conidia prevailed, but this fluctuation is constant even if 5-septate conidia are transferred separately. In other words, strains can not be grown with only 5-septate conidia by repeated selection of 5-septate conidia from a fungus with a normal fluctuation of 3- to 5-septate conidia.

In overwatering *Hypomyces ipomoeae*, its septate mature conidia swell and germinate if sufficient food allows further vegetative growth. If the medium is very dilute so that macroconidia can not be formed, microconidia will take their place (Pl. XIII, fig. *F*). While in almost pure water chlamydospores similar to those known in the section Martiella of Fusarium appear on hyphæ (Pl. XIII, fig. *D*, 1-3) or within conidia (conidio-chlamydospore, Pl. XIII, fig. *D*, 4). A transfer to a better medium, such as potato tuber or stems of legumes, will be helpful to produce perithecia. The young perithecium illustrated in Plate XIII, figure *E*, originated from a side branch of a proliferous hypha. The same spiral rolling up can be seen in Neocosmospora. The fungus requires 10 to 14 days for the mature red perithecia (Pl. XIII, fig. *C*) to disseminate their ascospores. If in the meantime cross sections are made from perithecia in various stages of their development, the asci and their remarkable variations from cylindrical to clavate forms can be followed (Pl. XIII, fig. *B*). Paraphyses appear occasionally, but in many preparations they are invisible, owing to their fragility and scarcity. Perithecia may be illustrated with prevalently cylindrical asci, with the clavate form, and with mixtures of both types, depending on the water content of the culture and other conditions. The shape of the asci is so modified by the elasticity of their membrane that this character does not seem to be of taxonomic importance. Water influences the outline of ascospores. Barrel-like, swollen cells (Pl. XIII, fig. *A*, 3) indicate either overmaturity in the presence of too much water or the stage before germination. If the medium is exhausted with water still present, a separation of the two cells can often be seen (Pl. XIII, fig. *A*, 4). This resembles Hypocrea. A slow desiccation of the culture performed after the maturity of the perithecia prevents a swelling of the ascospore and normal mature spores of ellipsoidal shape will prevail (Pl. XIII, fig. *A*, 1). Complete dryness decreases the turgescence, so that mature cells become conical with rounded top. Immature spores, however, may look more pointed then

because their delicate membrane can not withstand the increasing pressure to the same extent.

The influence of water has to be fully studied in order to understand these morphologic changes. The peridium of perithecia may be almost smooth when the lack of water prevents further growth of its cells. If the water and food supply, however, allow further development, protuberance-like projections will show the permanent activity of the peripherical cell complications. Also the periphyses will prolongate and form a compact cone adorning the perithecium with a beak. This beak is almost colorless and contrasts therefore with the red color of the peridium (Pl. XV, fig. *A*). The ostiolum at the top of the beak accumulates the ascospores as soon as the gradual drying out of the medium exerts a pressure on the perithecium. The peridial cells dry out and shrink, pressing the content of the ascus ball through the throat out of the ostiolum. Here they accumulate in a brown mass (Pl. XV, fig. *A*, 2 and 4). If the ostiolum is closed so completely that a higher pressure is required, the ejaculation is more explosive and the ascospores are shot out to several millimeters distance. This agrees exactly with the description given by Dr. Erwin F. Smith for Neocosmospora. Also, the stroma of our fungus is as variable in every respect as in Neocosmospora. It may be reduced to a minimum or grown as a thallus-like effuse layer.

*Hypomyces ipomoeae* may be a good example for discussing taxonomic difficulties. The diagnosis of this sweet-potato fungus, based on pure cultures, agreed closely with that of *Nectria coffeicola* Zimmermann (1901), a saprophyte on Coffea and Theobroma, also with *Nectria Goroschankiniana* Wahrlich (1886) from roots of *Vanda tricolor*, to some extent with *Nectria cancri* Rutgers (1913) from Theobroma. All these fungi were well illustrated by the authors, but mostly from field material. The differences shown in the following list between measurements from different authors will be understood from a discussion of their origin.

There are two methods of measuring spores, one of which gives the absolute size, the other the average size; the former comprises a much larger fluctuation of the spore size than the latter. The absolute fluctuation, therefore, is likely to include young and immature spores, which are smaller and less characteristic than normal mature spores. This is especially the case when the relations between size and age and septation are neglected. Immature ascospores may be comparatively small, 3 to 5$\mu$ in diameter, but broader, 5 to 6$\mu$, in maturity, and still broader, 6 to 8$\mu$, in germination or from overwatering. The absolute fluctuation, 3 to 8$\mu$, is too large to be of any taxonomic value, but the fluctuation of the average breadth, 4.5 to 6$\mu$, has some value because it reduces in a measurable way the limits of the absolute fluctuation. This reduction can be performed by measuring repeatedly hundreds of spores and taking the average size of each hundred. With cultures in different ages,

other averages with deviations from the first series would be obtained. With the same fungus on other substrata we may get still more deviations. In summarizing all results, we get the fluctuations of the average size. Experience has shown that we do not need hundreds, but merely 10 spores from each different culture condition in order to get the normal fluctuation of the average size.

*Hypomyces ipomoeae* (Hals.) Wollenw. from roots of *Ipomoea batatas* gave in pure culture the following average fluctuation of various stages:

Culture 30 days old on steamed potato tuber:
Perithecia, 250 by 200$\mu$; ascospores, 11 by 5 to 5.5$\mu$; 3-septate conidia, 33 by 4.25$\mu$ (6 per cent); 4-septate, 42 by 4.75$\mu$ (1 per cent); 5-septate, 55 by 5.25$\mu$ (56 per cent); 6-septate, 61 to 5.5$\mu$ (31 per cent); 7-septate, 66 by 5.5$\mu$ (5 per cent); 8-septate (1 per cent).

Culture 30 days old on steamed potato tuber:
Perithecia, 365 by 302$\mu$; ascospores, 13 by 5.75$\mu$; 0-septate conidia, 12 by 3.5$\mu$ (30 per cent); 3-septate, 33 by 4.25$\mu$ (60 per cent); 5-septate, 49 by 4.75$\mu$ (10 per cent).

Culture 23 days old on wheat heads:
Perithecia, 296 by 253$\mu$; ascospores, 13 by 5$\mu$; 0-septate conidia, 12 by 3.25$\mu$; 3-septate, 36 by 4.5$\mu$; 5-septate, 60 by 5.25$\mu$ (90 per cent); 6-septate, 66 by 5.25$\mu$.

Culture 45 days old on cotton stem:
Perithecia, 290 to 338 by 197 to 223$\mu$; ascospores, 12 by 5$\mu$; 3-septate conidia, 35 by 4.25$\mu$ (38 per cent); 4-septate (35 per cent); 5-septate, 59 by 4.5$\mu$ (25 per cent); 6-septate, 58 by 5$\mu$ (1 per cent).

Culture 14 days old on straw; overwatered:
Perithecia not measured; 5-septate conidia, 10 by 5.5$\mu$ (100 per cent).

Culture 14 days old on boiled rice:
Perithecia absent; conidia unicellular, 6 to 10 by 3 to 4.75$\mu$.

Culture 20 days old on sterile water:
Chlamydospores, 7 to 10$\mu$; originated from conidia.

The average size was higher in the presence of water than under dry conditions, but the number of septations could occasionally be decreased by overwatering. This table shows the fluctuation of average size and percentage of equiseptate conidia, indicating that 5-septate conidia prevail, being closely followed by 3-septate ones. Of all conidia 31 per cent may be 6-septate, 10 per cent 7-septate, 1 per cent 8-septate, but this is very seldom, and 3- and 5-septate spores will always be predominant in maturity. Young cultures bear more unicellular than septate conidia. The average size of equiseptate conidia is more constant than that of unequiseptate conidia. The perfect form of the fungus offers less difficulties, although fluctuations in the average size of perithecia may be considerable. Ascospores fluctuate but little, except when immature spores are included in the measurements. These measure 3 to 5.75$\mu$ in diameter, while mature ascospores average from 4.5 to 5.75$\mu$.

The following list of ascomycetes shows the relationship to *Hypomyces ipomoeae:*

*Nectria Goroshankiniana* Wahrlich (1886):

Perithecia, 360 by 320$\mu$,[1] ascospores, 12 to 15 by 4 to 5$\mu$; conidia (subnormal), 20 to 30 by 3.3 to 4.4$\mu$; chlamydospores described, but their connection with the perfect form unproved.

*Nectria coffeicola* Zimm. (1901):

Perithecia, 300 to 400$\mu$; ascospores, 10.5 to 13.5$\mu$; 3- to 5-septate conidia, 40 to 50 by 5$\mu$, chlamydospores (?)

*Hypomyces ipomoeae* (Hals.) (1892) Wollenw. (1913c):

Perithecia, 200 to 425 by 150 to 350$\mu$ (absolute fluctuation); ascospores, 8 to 16 by 3 to 8$\mu$ (absolute fluctuation), 3- to 5-septate conidia, 30 to 70 by 3.75 to 5.5$\mu$ (average fluctuation); chlamydospores, 7 to 10$\mu$.

*Nectria cancri* Rutg. (1913):

Perithecia, 400 to 500 by 300 to 400$\mu$; ascospores, 10 to 13 by 3 to 5$\mu$; 3- to 5-septate conidia, 30 to 60 by 3 to 5$\mu$; chlamydospores (?)

The illustrations of these fungi agree closely in the general appearance, but in the size of perithecia, ascospores, and conidia differences are observed. Some of these may be due to the special condition under which the fungus was collected or grown; others may be constant. The fact that fungi looking alike at the first glance will be proved different in pure culture became evident to the writer when he found on the taproot of *Cannabis sativa* a fungus with perithecia resembling those of *Hypomyces ipomoeae*. When this organism was studied in pure culture for a year, measurements of all spore stages were made. As is seen in the diagnosis, this hemp fungus developed larger perithecia and ascospores than *H. ipomoeae*. Its conidia, however, were never predominantly 5-septate, while in *H. ipomoeae* as many as 100 per cent of them can be found. The perithecia appeared slowly in the hemp fungus 20 to 30 days after the culture was started, while *H. ipomoeae* required only 10 to 15 days. Attempts to reduce these differences in size (Pl. XV, figs. *A*, *B*, and *C*), septation (Pl. XIII, figs. *G* and *J*), and rapidity of growth failed. One fungus could not be transformed into the other, although their relationship seems to be beyond question.

Since *Nectria cancri* has the size of the hemp perithecia and the normal mature ascospores illustrated by Rutgers are broader than his diagnosis (10 to 13 by 3 to 5$\mu$) gives, the hemp fungus may be provisionally determined as *Hypomyces cancri* (Rutg.), n. comb. (= *Nectria cancri* Rutg.). The fact that chlamydospores are not mentioned by Rutgers does not detract from this conclusion. The writer grows chlamydospores by overwatering the culture, but this method may not yet be well known.

The remarkable variation in the shape of the neck is illustrated in Plate XV, figs. *B* and *C*, and reminds us of *Nectria moschata* Glück. Plate XV, fig. *B*, shows two perithecia with a stromatic base, which is lacking in the other illustrations.

[1] Measurements of the size lacking in the diagnosis have been derived approximately from illustrated perithecia.

## III. GIBBERELLA Sacc.

### Gibberella Saubinetii (Mont.) Sacc.

*Gibberella Saubinetii* (Mont.) Sacc., 1879, *in* Michelia, v. 1, no. 5, p. 513.
*Gibberella cyanogena* (Desm.) Sacc., 1883, Syll. Fung., v. 2, p. 555.
*Gibberella Saubinetii* (Mont.) Sacc., Sorokine, 1890, *in* Trudy Obshch. Estestv. I. Kazansk. Univ., t. 22, vyp. 3, 32 p., 1 pl.
*Gibberella Saubinetii* (Mont.) Sacc., Selby, 1898, *in* Ohio Agr. Exp. Sta. Bul. 97, p. 40–42, fig. 4.
*Gibberella tritici* P. Henn., 1902, *in* Hedwigia, Bd. 41, Heft 6, p. 301.
*Fusarium roseum* autorum.
*Fusarium rostratum* App. and Wollenw., 1910, *in* Arb. Biol. Anst. f. Land- u. Forstw., Bd. 8, Heft 1, p. 30.
*Fusarium tropicalis* Rehm, 1898, *in* Hedwigia, Bd. 37, Heft 4, p. 194, is probably a synonym of *G. Saubinetii*.

Diagnosis.—Perithecial stage: Perithecia scattered or gregarious, ovoid to subconical free on the surface of the host as well as embedded in mycelium, or on a tubercular plectenchymatic stroma, which may either push in spærostilbe-like bodies through the surface of the host or remain endophytic, 150 to 250 by 100 to 250$\mu$. Peridium smooth and small celled at the basal part, but large-celled, verrucose occasionally, with protuberancelike projections of cell groups near the apical end, black to the unaided eye (turning red brown with acid reaction), dark blue with transmitted light except the almost colorless often rather prominent beak; asci up to over a hundred in each perithecium, intermixed with a few celled paraphyses; ascospores, 8 in one row or irregularly in two rows, subdorsiventral, fusiform slightly curved, tapering at the ends, ochreous in masses; largely 3-septate, 20 to 30 by 3.75 to 4.25$\mu$ (up to 5$\mu$ diameter in germination, indicated by a constriction at the septa).

Conidial stage.—In shape the conidia resemble the section Discolor of Fusarium, and are closely related to *Fusarium culmorum*, but differ in being longer, more slender, and less developed in septation; conidia 3- to 5-septate, 30 to 60 by 4.75 to 5.50$\mu$, ochreous in mass. Plectenchyma often carmine red, turning yellow in the presence of acid. No true chlamydospores. *F. culmorum*, on the other hand, has chlamydospores in intercalated chains and clusters.

Habitat.—This description is made from a strain isolated from a wheat kernel that failed to germinate (Dahlem, near Berlin, 1909). The following distribution is based on comparative pure-culture studies of fungi isolated from various hosts from different regions. It was found widely distributed within the Temperate Zone, causing scab disease of different kinds of cereals, especially wheat, emmer, rye, oats, spelt, and corn in Germany, Russia, Italy, and probably elsewhere. It has been isolated from berries of *Solanum tuberosum* at Friedenau, near Berlin, Germany, by the writer, and from sweet potatoes (*Ipomoea batatas*) in storage by Mr. C. A. Ludwig, Lafayette, Ind.

According to Saccardo (Michelia, v. 1, no. 5, p. 513, 1879) the fungus also occurs on dead stems of Conium, Phytolacca, Cannabis, Curcurbita, Convolvulus, Clematis, Beta, Angelica, Stipa, Gyneria, Asparagus, and Scirpus, and on branches of Gleditschia, Rosa, Robinia, Juglans, Fraxinus, Ulmus, Coronilla, Rubus, and Buxus in France, Italy, Germany, Austria, Great Britain, Spain, Belgium, Algeria, North America, and Australia. A. D. Selby, in his "Brief handbook of the diseases of cultivated plants" (Ohio Agr. Exp. Sta. Bul. 214, p. 454, 1910), adds clover (Trifolium) and alfalfa (Medicago) as new hosts. Some of these statements, however, seem to be merely based on the presence of the conidial stage, to which different names have been given, such as *Fusarium roseum* Link, *F. herbarum* (Corda) Fr., and *F. rostratum* App. and Wollenw.

This characteristic fungus, *Gibberella Saubinetii* (Pls. XIV, figs *D–G*, XVI, fig. *O*), is widely distributed on cereals. Sorokine (1890) and Selby (1898) illustrated it well and many authors described its life cycle. Since

all descriptions have been based on field material and not on pure cultures, a few supplementary notes and illustrations may be desirable in order to show how much the conidia of this fungus differ from some species of Fusarium often associated with them in the field, and how slender the ascospores are compared with those of *Gibberella pulicaris*, a fungus often confused with *G. Saubinetii*. Attention has already been given to these points in this paper, and the conduct of this fungus in pure culture has been freely discussed in "Criteria of the norm."

In Table I a record of some measurements is given. The conidia have a range of septation differing with age and substratum, but 3-to 5-septate conidia prevail under various conditions. The average size varies greatly if the septation is neglected, but equiseptate spores fluctuate in small limits only. If we measure the absolute fluctuation of the size, we get, of course, a wider limit. For instance, the absolute size of 3-septate conidia in a 40-day-old culture on steamed potato stems fluctuates from 21 to 39 by 3 to 5.5$\mu$ in one preparation, from 24 to 36 by 3.4 to 5.1$\mu$ in another, while the average size based on the average of 10 measurements was 30 by 4.25$\mu$. This fact proves an almost general law, which could be given in the rule "The absolute fluctuation of the spore size is a function of the average fluctuation." If 20 per cent of its value is subtracted from and added to 30$\mu$, the result is 24 and 36$\mu$. The same operation extended to 30 per cent gives 21 and 39$\mu$. The breadth, 4.25, treated in the same way gives 3.4 and 5.1$\mu$ in one, 3 and 5.5$\mu$ in the other case. When the fluctuation of the average size of 3-septate conidia has been obtained, 30 to 46 by 4.25 to 4.5$\mu$, and desire an approximate idea of the absolute fluctuation, the mean proportional, which is 38 by 4.375$\mu$, is taken, 30 per cent added and subtracted, and about 27 to 49 by 3 to 5.75$\mu$ is obtained, which corresponds almost with the fact. For this reason the absolute fluctuation, given by Appel and Wollenweber (1910) is left out as superfluous in the diagnoses of this paper.

TABLE I.—*Fluctuation of the average sizes of the conidial and perfect stages of Gibberella Saubinetii based on the average of 10 measurements*

CONIDIAL STAGE

| Age of culture. | Pure culture on sterilized— | Average length, breadth, and percentage of equiseptate conidia. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 to 2-septate. | | 3-septate. | | 4-septate. | | 5-septate. | | 6-septate. | | 7-septate. | |
| *Days.* | | $\mu$ | P. ct. | $\mu$ | P. ct. | $\mu$ | P. ct. | $\mu$ | P. ct. | $\mu$ | P. ct. | $\mu$ | P. ct. |
| 7.... | Potato stem | ..... | .... | 36 by 4.5 | 18 | ..... | 11 | 50 by 5.25 | 70 | ..... | 1 | ..... | .... |
| 12.... | .....do..... | ..... | .... | 37 by 4.5 | 30 | ..... | 19 | 52 by 5 | 40 | ..... | 4 | ..... | 1 |
| 30.... | .....do..... | ..... | .... | 33 by 4.5 | 8 | 43 by 5 | 23 | 45 by 5 | 69 | ..... | .... | ..... | .... |
| 40.... | .....do..... | ..... | 2 | 30 by 4.25 | 68 | ..... | 22 | ..... | 8 | ..... | .... | ..... | .... |
| 3.... | Potato tuber | ..... | 12 | 37 by 4.50 | 50 | ..... | 12 | 47 by 4.75 | 21 | ..... | 3 | ..... | 2 |
| 7.... | Potato tuber (pionnotes). | ..... | 9 | 46 by 5 | 38 | ..... | 19 | 60 by 5.5 | 27 | 64 by 5.7 | 6 | 71 by 5.75 | 1 |
| 6.... | Wheat straw | ..... | .... | ..... | .... | ..... | .... | 56 by 5.25 | .... | ..... | .... | ..... | .... |
| 6.... | Nutrient agar | ..... | 7 | ..... | 29 | ..... | 24 | 54 by 5.5 | 23 | ..... | 10 | ..... | .... |
| 40.... | Wheat kernels. | ..... | .... | 35 by 4.25 | .... | 45 by 4.5 | .... | 49 by 4.75 | .... | ..... | .... | ..... | .... |
| 40.... | Rice | ..... | 3 | 32 by 4.5 | 55 | 41 by 4.75 | 26 | 45 by 4.75 | 16 | ..... | .... | ..... | .... |

TABLE I.—*Fluctuation of the average sizes of the conidial and perfect stages of Gibberella Saubinetii based on the average of 10 measurements*—Continued

PERFECT STAGE

| Age of culture. | Pure culture on sterilized— | Height and diameter of perithecia. | Average size and percentage of equiseptate ascospores. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1-septate. | | 2-septate. | | 3-septate. | |
| *Days.* | | μ | μ | *P. ct.* | μ | *P. ct.* | μ | *P. ct.* |
| 30.... | Potato stem | 214 by 183 | 21 by 3.5 | 36 | 22 by 3.5 | 13 | 22 by 3.75 | 51 |
| 30.... | Vicia faba stem | 225 by 190 | ..... | ..... | ..... | ..... | 25 by 4 | 100 |
| 60.... | .....do | 200 by 170 | ..... | ..... | ..... | ..... | 29 by 4 | 100 |
| 60.... | Wheat straw | 178 by 130 | 15 by 4 | 9 | 18 by 4 | 5 | 18 by 4.25 | 86 |
| 40.... | Cotton stem | 245 by 230 | ..... | ..... | ..... | ..... | 24 by 4 | 100 |

Plate XIV contains illustrations from Gibberella, the original strain from a wheat kernel which failed to germinate. These kernels have a carmine color when the subcuticular plectenchyma (Pl. XIV, fig. *J*) is well developed. Red is formed as the basic modification of the fungus, while yellow is the acid modification, which can be observed on steamed rice in pure culture. *Fusarium subulatum*, *F. culmorum*, and *F. metachroum* have the same color shades and are also common on cereals, so that the red grains are not due alone to the presence of Gibberella. The perithecia of this fungus, being blue, as a rule, turn red brown with the addition of an alkali. On steamed potato tuber the conidia form a short-lived pionnotes, which is brownish white to ochreous, depending on the moisture and the influence of the carmine, which enters the conidia to some extent. The conidia of this pionnotes rapidly swell (Pl. XIV, fig. *H*), separate into cells, germinate, and produce new conidia (Pl. XIV, fig. *K*), which anastomose and form a stroma, while in the other species mentioned the conidia remain perfect, dry out, and are long-lived. It was interesting to note the increase of septa in germinating spores, which may have as many as 9 (Pl. XIV, fig. *H*), while the normal conidia (Pl. XIV, figs. *G* and *F*) have 3 to 5 septa. The ascospores swell (Pl. XIV, fig. *E*, 1) like the conidia, and this swelling often lasts even after desiccation. However, cultures on straw may develop a type of ascospores with a smooth outline (Pl. XIV, fig. *E*, 2) unless a rapid desiccation causes constriction between the septa (Pl. XIV, fig. *E*, 3). A hundred asci may be formed in one perithecium, but, as a rule, they are not so numerous. Typical paraphyses are seldom seen between the asci, but they are present (Pl. XIV, fig. *D*) and are 4 to 6 celled. Perithecia have two sizes of cells. Groups of large cells surround the ostiolum like a collar, which may or may not be pronounced (Pl. XIV, fig. *C*). Two such collars rarely seen in other perithecia (Pl. XIV, fig. *A*) proved the fact that sometimes two ostioli allow the ascospores to escape. The main body of the peridium is small celled, and the arrangement of the cells indicates their hyphal origin. A longitudinal section shows the peridium to consist of three layers (Pl. XIV, fig. *B*). The stroma of Gibberella is very

changeable. It may be reduced to a few hyphæ or formed as a thallus-like layer. On substrata, such as Robinia stems, a sphærostilbe-like stroma may be formed with a colony of perithecia at the top (Pl. XV, fig. *D*), or a short but compact stroma develops with a single perithecium (Pl. XV, fig. *G*) or a few together. The protuberancelike projections on the peridium are often not confined to the collar surrounding the neck or the ostiolum. They may develop on any place, especially when moisture allows a continuous growth beyond the time of maturity of the spores. These projections (Pl. XV, figs. *D* and *E*) resemble *Hypomyces ipomoeae*, but all stages from a verrucose to a smooth peridium (Pl. XV, fig. *F*) can be secured by selection of media and special methods of transfers in connection with various amounts of moisture. A larger stroma develops when mycelium is used in starting the culture on a cotton or Melilotus stem. But very few colonies of perithecia may appear with this method. Conidia and ascospores transferred to the same substratum will produce more numerous perithecia with less stroma. The illustrations in Plate XV, figures *D–G*, are made from Gibberella, the strain isolated from *Ipomoea batatas;* but the strain isolated from Triticum (Pl. XIV) corresponds in all respects with the sweet potato strain. In brief, these pure cultures show constancy in ascospores and conidia, but so much variation in the production of stroma and general appearance of perithecia that field material with such differences would be easily referred to more than one species, or even genus.

## KEY TO THE SPECIES OF FUSARIUM DESCRIBED FROM PURE CULTURES GROWN IN DAYLIGHT

### A. SPECIES OF FUSARIUM WITHOUT KNOWN PERFECT FORM

I. Terminal chlamydospores present.

a. Conidia cream-colored to brownish white, except in *Fusarium coeruleum* (Lib.) Sacc.; conidia not sharply pointed at the ends; foot and heel of the base reduced to a papilla-like appendage. No wine-red color on sterilized, watered rice. Section Martiella .....(Pl. XVI, fig. *K*) *Fusarium radicicola*, n. sp.

b. Conidia ochreous to salmon colored, except in *Fusarium redolens* Wollenw.; conidia with curved apical end constricted like a flask neck and with a pedicellate base, but without a prominent heel. A wine-red color, turning blue upon the addition of alkali on sterilized watered rice, except in *F. conglutinans* Wollenw. Section Elegans.

1. Sickle-shaped conidia; slender, about 11 to 13 times longer than broad.

*a*. Sporodochia absent, conidia mostly unicellular.
*Fusarium orthoceras* App. and Wollenw.

*b*. Sporodochia present; 3-septate conidia up to 100 per cent.

* Blue sclerotial plectenchymata effuse and few on sterilized potato tuber.
(Pl. XVI, fig. *N*) *Fusarium orthoceras*, var. *triseptatum*, n. var.

** Blue sclerotial plecten chymata small, convex, numerous on sterilized potato tuber.....(Pl. XVI, fig. *D*) *Fusarium batatatis*, n. sp.

2. Sickle-shaped conidia 8–9 times longer than broad.

*a*. Pionnotes reduced.....*Fusarium oxysporum* Schlecht.

*b*. Pionnotes perfect.....(Pl. XVI, fig. *F*) *Fusarium hyperoxysporum*, n. sp.

II. Terminal chlamydospores absent, conidia ochreous to salmon colored.
  a. Conidia with curved apical end constricted like a flask neck, heel of the pedicellate base not prominent. Section Discolor.
    1. Intercalated chlamydospores rare and not in clusters. No carmine color on sterilized potato tuber. .(Pl. XVI, fig. *L*) *Fusarium incarnatum* (Rob.) Sacc.
    2. Intercalated chlamydospores occur singly and in cluster chains. A carmine color develops on sterilized potato tuber.
(Pl. XVI, fig. *J*) *Fusarium culmorum* (W. G. Sm.) Sacc.
  b. Conidia with a prolongated and pointed apical end and with the heel of the pedicellate base prominent.
    1. Parabolic to hyperbolic curves prevail in conidia seen in side view. No carmine color on steamed potato tuber. Section Gibbosum.
      *a*. Apical end of conidia curved, subfiliform.
(Pl. XVI, fig. *P*) *Fusarium caudatum*, var. *volutum*, n. var.
      *b*. Apical end of conidia curved, filiform.
(Pl. XVI, fig. *M*) *Fusarium caudatum*, n. sp.
III. Terminal and intercalated chlamydospores absent. Conidia resemble those in the section Gibbosum, but hyperbolic curves are seldom pronounced. A carmine mycelium color on steamed potato tuber. Section Roseum.
(Pl.XVI, fig. *G*) *Fusarium acuminatum* Ell. and Ev.

B. SPECIES OF FUSARIUM WITH KNOWN PERFECT FORM

1. Conidial stage similar to the section Martiella of the genus Fusarium, but with a subpedicellate base. Section Pseudomartiella of the genus Hypomyces.
  *a*. Conidia largely 3-septate. Perithecia averaging in size 350 to 450 by 275 to 375$\mu$. Ascospores, 10 to 15 by 5 to 6.75$\mu$.
(Pl. XIII, fig. *J*) *Hypomyces cancri* (Rutg.) n. comb.
  *b*. Conidia largely 5-septate. Perithecia, 225 to 375 by 175 to 300$\mu$. Ascospores, 10 to 13 by 4.5 to 6$\mu$.
(Pl. XVI, fig. *H*) *Hypomyces ipomoeae*, (Hals.) Wollenw.
2. Conidial stage similar to the section Discolor of the genus Fusarium, but chlamydospores absent. Genus Gibberella.
(Pl. XVI, fig. *O*) *Gibberella Saubinetii* (Mont.) Sacc.

This key might have been based entirely upon the morphological characters and curvature of the conidia, but since the color reactions offer a simpler, though less trustworthy means of identification, they have been employed. The key, therefore, should be regarded only as an aid in identification, not as a guide to the morphology, which has been discussed in the diagnosis and illustrated in detail in the illustrations.

In Table II the average size of the various spore types has been given approximately to allow a survey of the differences between the species. The spore diameter is an important factor for the determination of species of Fusarium, as may be seen in comparing equiseptate conidia of *Fusarium culmorum* and *F. camptomctachroum*. The conidial length is less significant. The measurements of chlamydospores recorded are confined to their cross diameter, the two dimensions not varying much in the almost spherical unicellular spores. Ovoid and 2-celled spores have a major axis which consequently has a higher average length than the diameter of spherical spores shows in Table II.

The color range indicates that related species have similar colors of corresponding organs. The relations of color and reaction of organisms, studied also by Milburn (1904) and Bessey (1904), are constant under constant conditions. On substrata rich in carbohydrates many fungi and bacteria produce alkaline substances; on those rich in peptone, acid substances. Each reaction is accompanied by special colors, which change with the change of reactions of the substratum. Many fungi, however, forced to grow on very alkaline or acid media refuse to develop characteristic colors.

Table II, therefore, does not refer to artificially made acid or alkaline media, but to reactions developed by the fungi on potatoes, rice, and stems sterilized after the addition of water, but otherwise unchanged. The reaction of these media differs but slightly from neutral.

Blue perithecia of Gibberella turn red to brown, and carmine mycelium turns yellow with acids, but their original color redevelops by addition of sufficient alkali. This alternative change of color can be produced repeatedly. The yellow color on rice turns violet with alkali and redevelops yellow with acids. These relations between reaction and color are so constant that they facilitate the determination of many species and are also of value for the characterization of sections.

TABLE II.—*Some characters of the described species of Fusarium, with and without known ascus stage, occurring on sweet potato*

| Name of fungus. | Section. | Perithecia. | | | | Ascospores. | | | | Average diameter of chlamydospores. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Presence or absence. | Color modification. | | Average size. | Septation. | Shape. | | Average size. | Mostly terminal and intercalated 1 to 2 cellular. | Mostly intercalated in chains and clusters. |
| | | | Basic. | Acid. | | | Ellipsoidal. | Fusoid-falcate. | | | |
| Hypomyces ipomoeae (Hals.) Wollenw. | Pseudomartiella... | *a* + | Red... | Yellow.. | 225 to 375 by 175 to 300 μ | 1 | *a* + | *b* − | 10 to 13 by 4.5 to 6 μ | 7 to 10 μ | ..... μ |
| Hypomyces cancri (Rutg.), n. comb. | ..... | *a* + | Red... | Yellow.. | 350 to 450 by 275 to 375.. | 1 | *a* + | *b* − | 10 to 15 by 5 to 6.75..... | 7 to 10 | ..... |
| Gibberella Saubinetii (Mont.) Sacc. | ..... | *a* + | Blue.. | Red..... | 150 to 250 by 100 to 250.. | 3 | *b* − | *a* + | 20 to 30 by 3.75 to 4.25... | ..... | ..... |
| Fusarium acuminatum Ell. and Ev. | Roseum..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Fusarium culmorum (W. G. Sm.) Sacc. | Discolor..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 14 |
| Fusarium incarnatum (Rob.) Sacc. | .....do..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 14 |
| Fusarium caudatum, n. sp..... | Gibbosum..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 14 |
| Fusarium caudatum, var. volutum, n. var. | .....do.. | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 14 |
| Fusarium orthoceras App. and Wollenw. | Elegans..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 10 | ..... |
| Fusarium orthoceras triseptatum, n. var. | .....do..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 10 | ..... |
| Fusarium batatatis, n. sp..... | .....do..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 10 | ..... |
| Fusarium oxysporum Schlecht.. | .....do..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 10 | ..... |
| Fusarium hyperoxysporum, n. sp. | .....do..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 10 | ..... |
| Fusarium radicicola, n. sp..... | Martiella..... | *b* − | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 to 10 | ..... |

*a* +, Present. *b* −, Absent.

TABLE II.—*Some characters of the described species of Fusarium, with and without known ascus stage, occurring on sweet potato*—Continued

| Name of fungus. | Characteristic color. | | | | Conidia. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blue sclerotial plecten-chymata. | Modification of plectenchymatic mycelium. | | Conidia in masses. | Normal septa-tion. | Average size of 3-septate conidia. | Maximal percentage of normal equiseptate conidia. | | | Average size of 5-septate conidia. |
| | | Basic. | Acid. | | | | 3-septate. | 4-septate. | 5-septate. | |
| | | | | | | μ | | | | μ |
| Hypomyces ipomoeae (Hals.) Wollenw. | *b* — | Olive green to brown | Red | Brownish white | 5 (3 to 5) | 30 to 45 by 3.75 to 5 | 60 | 35 | 100 | 45 to 70 by 4.25 to 5.5. |
| Hypomyces cancri (Rutg.), n. comb. | *b* — | do | do | do | 3 (3 to 5) | 30 to 45 by 3.75 to 5 | 100 | 50 | 30 | 45 to 55 by 4.25 to 5.5. |
| Gibberella Saubinetii (Mont.) Sacc. | *b* — | Red | Yellow | Ochreous to salmon. | 3 to 5 | 30 to 45 by 4.25 to 5 | 100 | 25 | 100 | 45 to 60 by 4.75 to 5.5. |
| Fusarium acuminatum Ell. and Ev. | *a* + | do | do | Orange | 5 | 20 to 45 by 2.75 to 4.25 | 15 | 35 | 100 | 40 to 70 by 3 to 4.5. |
| Fusarium culmorum (W. G. Sm.) Sacc. | *b* — | do | do | Ochreous to salmon. | 5 | 25 to 35 by 5 to 6 | 30 | 30 | 100 | 30 to 45 by 5.5 to 7. |
| Fusarium incarnatum (Rob.) Sacc. | *b* — | Brown | Brown | do | 3 to 5 | 20 to 25 by 3.5 to 4.5 | 100 | 20 | 100 | 30 to 50 by 3.75 to 5. |
| Fusarium caudatum, n. sp | *b* — | do | do | do | 5 | 25 to 35 by 2.75 to 4 | 10 | 25 | 100 | 40 to 80 by 3 to 4.5. |
| Fusarium caudatum, var. volutum, n. var. | *b* — | do | do | do | 3 (3 to 5) | 25 to 35 by 2.5 to 3.75 | 100 | 50 | 30 | 30 to 50 by 3 to 4. |
| Fusarium orthoceras App. and Wollenw. | *b* — | Blue | Red | do | 0 (3) | 25 to 46 by 3.25 to 4 | 15 | 3 | 1 | 40 to 50 by 3.25 to 4. |
| Fusarium orthoceras triseptatum, n. var. | *b* — | do | do | do | 3 (0) | 25 to 45 by 2.75 to 4 | 100 | 20 | 8 | 40 to 50 by 3.25 to 4. |
| Fusarium batatatis, n. sp | *a* + | do | do | do | 3 | 25 to 45 by 2.75 to 4 | 100 | 25 | 16 | 40 to 50 by 3 to 4. |
| Fusarium oxysporum Schlecht | *a* + | do | do | do | 3 | 25 to 40 by 3.25 to 4.5 | 100 | 25 | 10 | 32 to 42 by 3.5 to 4.75. |
| Fusarium hyperoxysporum, n. sp. | *a* + | do | do | do | 3 | 25 to 40 by 3.25 to 4.5 | 100 | 25 | 10 | 32 to 42 by 3.5 to 4.75. |
| Fusarium radicicola, n. sp | *b* — | Olive green to brown | do | Brownish white | 3 | 30 to 45 by 3.75 to 5 | 100 | 30 | 5 | 40 to 50 by 4 to 5.25. |

*a* +, Present. *b* —, Absent.

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SUMMARY

(1) Only 2 out of 11 species and 2 varieties of Fusarium on sweet potato (*Ipomoea batatas* Poir.) developed the perfect form: *Hypomyces ipomoeae* (Hals.) Wollenw. [= *Nectria ipomoeae* Hals. = *Creonectria ipomoeae* (Hals.) Seav.] and *Gibberella Saubinetii* (Mont.) Sacc.

(2) The other species of Fusarium on sweet potato remain in the genus Fusarium Lk., and belong to the sections Martiella, Elegans, Discolor, Gibbosum, and Roseum.

(3) *Fusarium orthoceras* App. and Wollenw., *F. oxysporum* (Schlecht.), *F. incarnatum* (Rob.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., and *F. acuminatum* Ell. and Ev. are common but not obligate sweet-potato fungi. The first two are especially prevalent. These five are also found on Solanum and other hosts. *F. oxysporum* is proved a cause of wilt disease of Solanum, but not of Ipomoeae.

(4) *Fusarium batatatis* Wollenw. and *F. hyperoxysporum* Wollenw. causing wilt disease on Ipomoea (according to Harter and Field) are species of the section Elegans. The former is related to *F. orthoceras*, the latter to *F. oxysporum*.

(5) *Fusarium culmorum* (W. G. Sm.) Sacc., synonym of *F. rubiginosum* App. and Wollenw., occurs more often on cereals, especially Triticum, than on Ipomoea and Solanum.

(6) Species of Fusarium described as new: *F. radicicola; F. orthoceras*, var. *triseptatum; F. batatatis; F. hyperoxysporum; F. caudatum;* and *F. caudatum*, var. *volutum*.

(7) New combination: *Hypomyces cancri* (Rutg.), n. comb. (= *Nectria cancri* Rutg.).

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## PLATE XII

Fig. A.—*Fusarium batatatis*, n. sp.: Mycelium stage with blue sclerotial plectenchymata on steamed potato tuber.

Fig. B.—*Fusarium batatatis*, n. sp.: Ochreous- to salmon-colored pionnotes on the same medium, but from a transfer of a single sickle-shaped conidium.

Fig. C.—*Fusarium batatatis*, n. sp.: Wine-red acid color modification of the fungus on steamed rice, turning blue with alkali.

Plate XII was reproduced from paintings made by Mr. J. M. Shull from 10-day-old test-tube cultures of the fungus.




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## PLATE XIII

Figs. A–G.—*Hypomyces ipomoeae* (Hals.) Wollenw. Fig. H.—*Fusarium incarnatum* (Rob.) Desm. Fig. J.—*Hypomyces cancri* (Rutg.), n. comb. The drawings of *H. ipomoeae* were made from subcultures on moist wheat heads of a strain isolated by Dr. Reddick, Cornell University Experiment Station, from a badly rotted sweet potato (*Ipomoea batatas*), sent him from Wooster, Ohio, on April 30, 1907, by J. M. van Hook of the Ohio State Agricultural Experiment Station. *F. incarnatum* is illustrated from a strain isolated from sweet potato.

Fig. A.—*Hypomyces ipomoeae.* Ascospores: 1, Ellipsoidal shape; 2, form of a double paraboloid; 3, overripe, slightly swollen stage; 4, separation of the two cells in overripe stage. × 1,000.

Fig. B.—*Hypomyces ipomoeae.* Asci with a paraphysis. × 500.

Fig. C.—*Hypomyces ipomoeae.* Perithecium. × 200.

Fig. D.—*Hypomyces ipomoeae.* Chlamydospores: 1 and 3, lateral; 2, intercalated and terminal; 4, intercalated within a conidium (conidiochlamydospore). × 1,000.

Fig. E.—*Hypomyces ipomoeae.* Perithecium formed by spiral coiling of a lateral hypha. × 500.

Fig. F.—*Hypomyces ipomoeae.* False conidial heads produced at the end (1) of conidiophores by spores suspended in drops of water. The conidiophore sprang from an old conidium which was separated into two parts, one of which was dead (*a*). × 500.

Fig. G.—*Hypomyces ipomoeae.* Normal conidia: 1, More curved at the ends than the other spores; 2–7, tri- to quinque-septate spores.

Fig. H.—*Fusarium incarnatum* (Rob.) Desm.: Conidia, *a*, short, *b*, slender; 1–3, normal 5-septate conidia; 4, lanceolate; 5, exceptionally large; *c*, pedicellate base without heel.

Fig. J.—*Hypomyces cancri* (Rutg.), n. comb.: Mature conidia, the first one (1) being especially large.

## PLATE XIV

*Gibberella Saubinetii* (Mont.) Sacc. This fungus was isolated from a wheat kernel in Dahlem, near Berlin, Germany. The first perithecia appeared in pure cultures after several transfers to fresh media. Thus far no differences have been observed between the wheat and the sweet-potato strains.

Figs. A–C.—*Gibberella Saubinetii:* Perithecia grown in pure culture; A, on stem of *Vicia faba,* with two ostiola surrounded by a collar of large peridial cells; B, on wheat grains showing the ascus ball after one-half of the peridium had been lifted by a longitudinal section; C, on Irish potato stem, without a distinct collar. × 200.

Fig. D.—*Gibberella Saubinetii:* Two asci with a paraphysis. ×500.

Fig. E.—*Gibberella Saubinetii:* Ascospores, 1, with slightly swollen cells, overripe; 2, normal shape; 3, dried condition. ×1,000.

Fig. F–G.—*Gibberella Saubinetii:* Normal conidia grown on Irish potato stem. F, 12 days old; G, 6 days old, culture watered more, therefore the conidia are broader than in figure F. ×1,000.

Fig. H.—*Gibberella Saubinetii:* Abnormally multiseptate conidium about to germinate. These conidia are frequently to be found with swollen cells in young, and sometimes old cultures on the parenchyma of potato tubers. (Compare fig. K.) ×1,000.

Fig. J.—*Gibberella Saubinettii:* Plectenchymatic parts of a stroma, formed by closely interwoven chains of swollen cells which have a thick membrane and brown to red contents with many vacuoles. Not to be confused with true chlamydospores, although very resistant to unfavorable conditions.

Fig. K.—*Gibberella Saubinetii:* A number of conidia formed in young cultures on the moist surface of potato cylinders. Septation (1, 3) and shape (2) rarely normal; 4, mother conidium broken into two halves (a, b), both of which have developed some small conidiophores from the cells torn asunder. ×500.

## PLATE XV

Fig. A.—*Hypomyces ipomoeae* (Hals.) Wollenw.: Sweet-potato strain, its perithecial stage isolated in 1907 by Dr. Donald Reddick, Cornell University Experiment Station. (See legend of Pl. XIII.) 1, 2, Grown in pure culture on cotton stem; 3, on maple stem; 4, on wheat straw; 5 and 6, on potato cylinder. ×50.

Fig. B–C.—*Hypomyces cancri* (Rutg.), n. comb.: The hemp strain found with perithecia on a dead taproot of hemp at the soil level, Potomac Flats, Washington, D. C., 1912. The perithecia and ascospores are larger, but the conidia are less septate than those of the sweet potato strain. B, Grown on steamed corn kernels. C, 1, Perithecia from the original field material; C, 2, 3, Grown in pure culture on cotton stem. ×50.

Fig. D–G.—*Gibberella Saubinetii* (Mont.) Sacc.: The mycelium stage was isolated in 1912 from sweet-potato tubers by Mr. C. A. Ludwig, Lafayette, Ind. The writer obtained perithecia after the second transfer of conidia on cotton stem, wheat straw and heads, potato stem, etc. The first perithecia appeared gregarious on a coremium-like or irregular plectenchymatic stroma. D, Grown on stem of Robinia, which was more or less reduced when single ascospores of these perithecia were transferred to fresh moist steamed stems of plants; F and G, grown on cotton stem (on almost dry wheat straw the stroma could be reduced still more and completely disappeared in some of the later cultures on this medium); E, grown on wheat straw.

## PLATE XVI

*Fusarium* spp. on sweet potato with and without known perfect stage, grown on sterilized vegetables.

Fig. A–E.—*Fusarium batatatis*, n. sp.: A, Microconidia. ×1,000. B, 1, Intercalated and terminal chlamydospores. 2, In young stage. 3, Branch from sclerotial plectenchymata, therefore no true chlamydospores. 4, Chlamydospores formed from the content of conidial cells (conidio-chlamydospores). 5, Mature chlamydospores. ×500. C, Two conidia anastomosing, one of them producing microconidia. ×500. D, Normal conidia from sporodochia. ×1,000. E, Conidiophore from a sporodochium. ×500.

Fig. F–P.—Characteristic conidia of different species of Fusarium. ×1,000.

Fig. F.—*Fusarium hyperoxysporum*, n. sp.

Fig. G.—*Fusarium acuminatum* Ell. and Ev.

Fig. H.—*Hypomyces ipomoeae* (Hals.) Wollenw.

Fig. J.—*Fusarium culmorum* (W. G. Sm.) Sacc.

Fig. K.—*Fusarium radicicola*, n. sp.

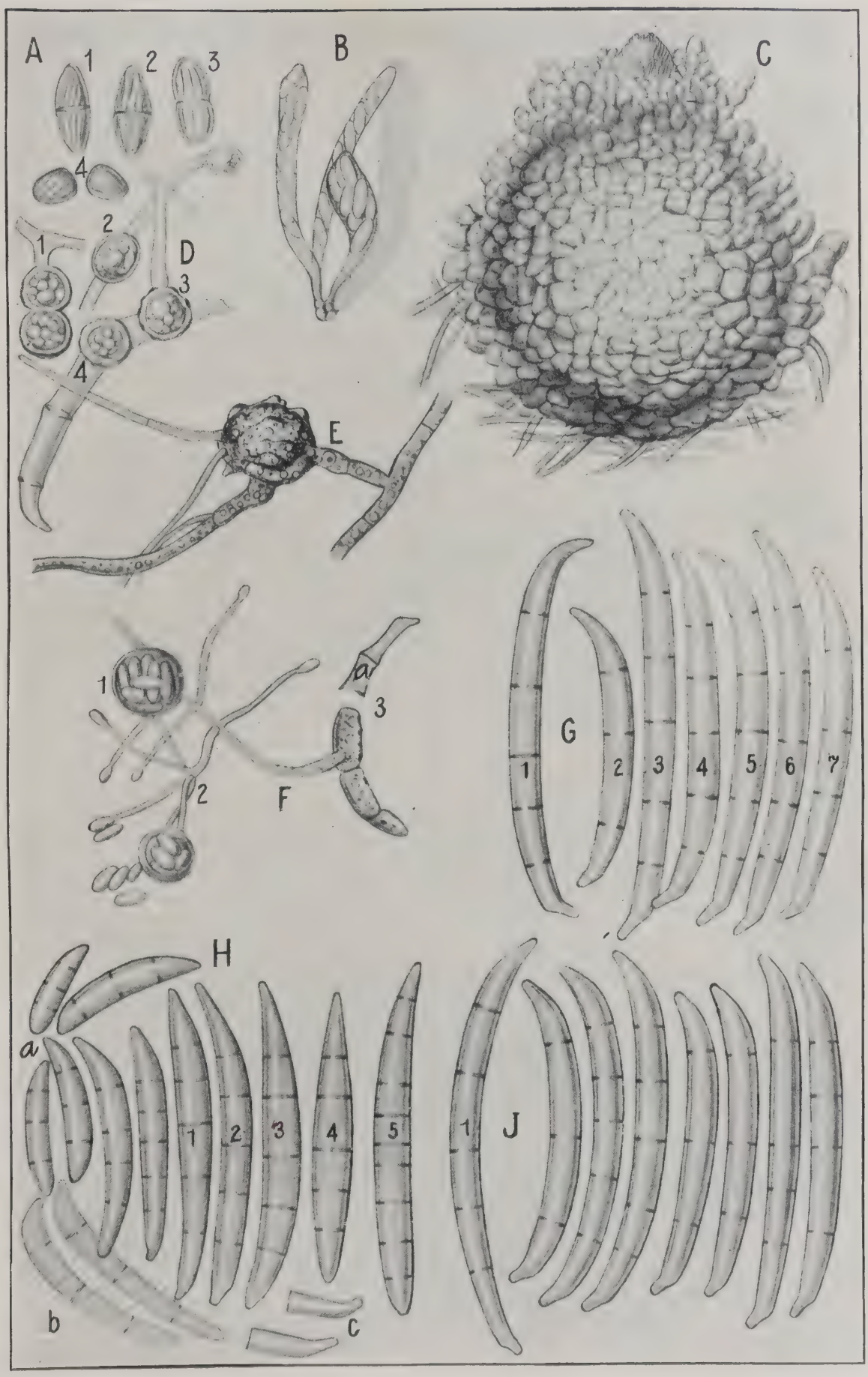
Fig. L.—*Fusarium incarnatum* (Rob.) Sacc.

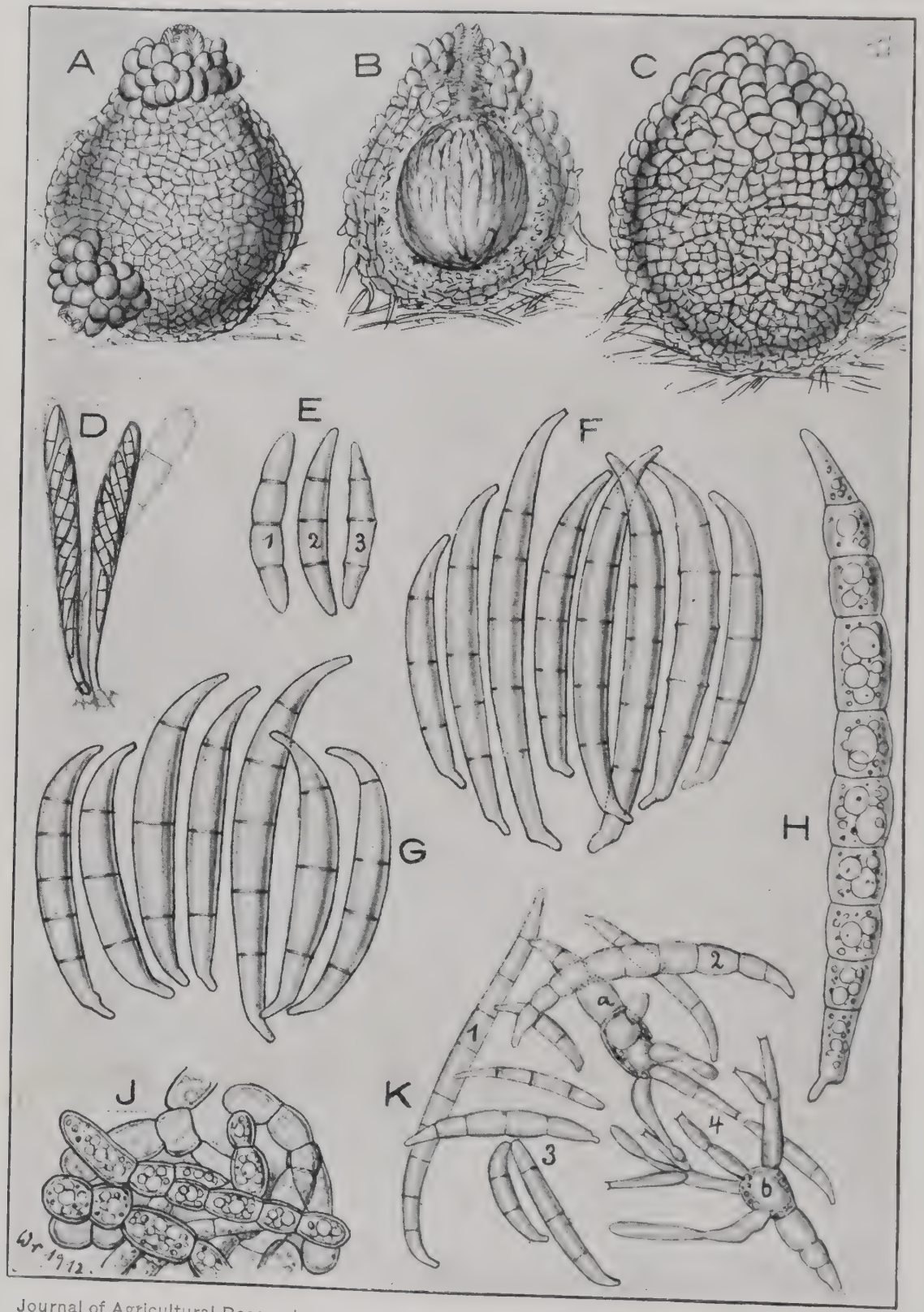
Fig. M.—*Fusarium caudatum*, n. sp.

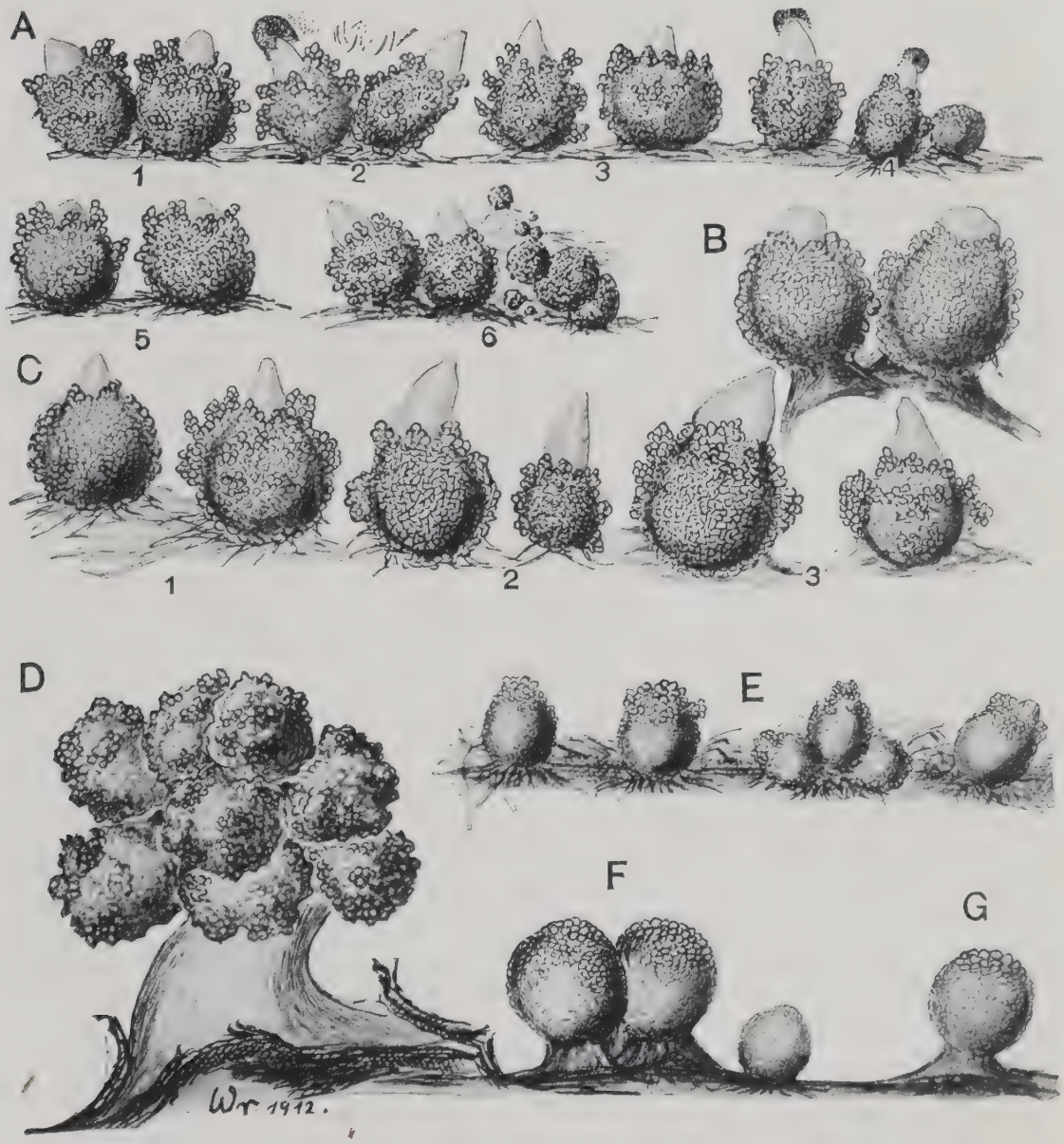
Fig. N.—*Fusarium orthoceras* var. *triseptatum*, n. var.

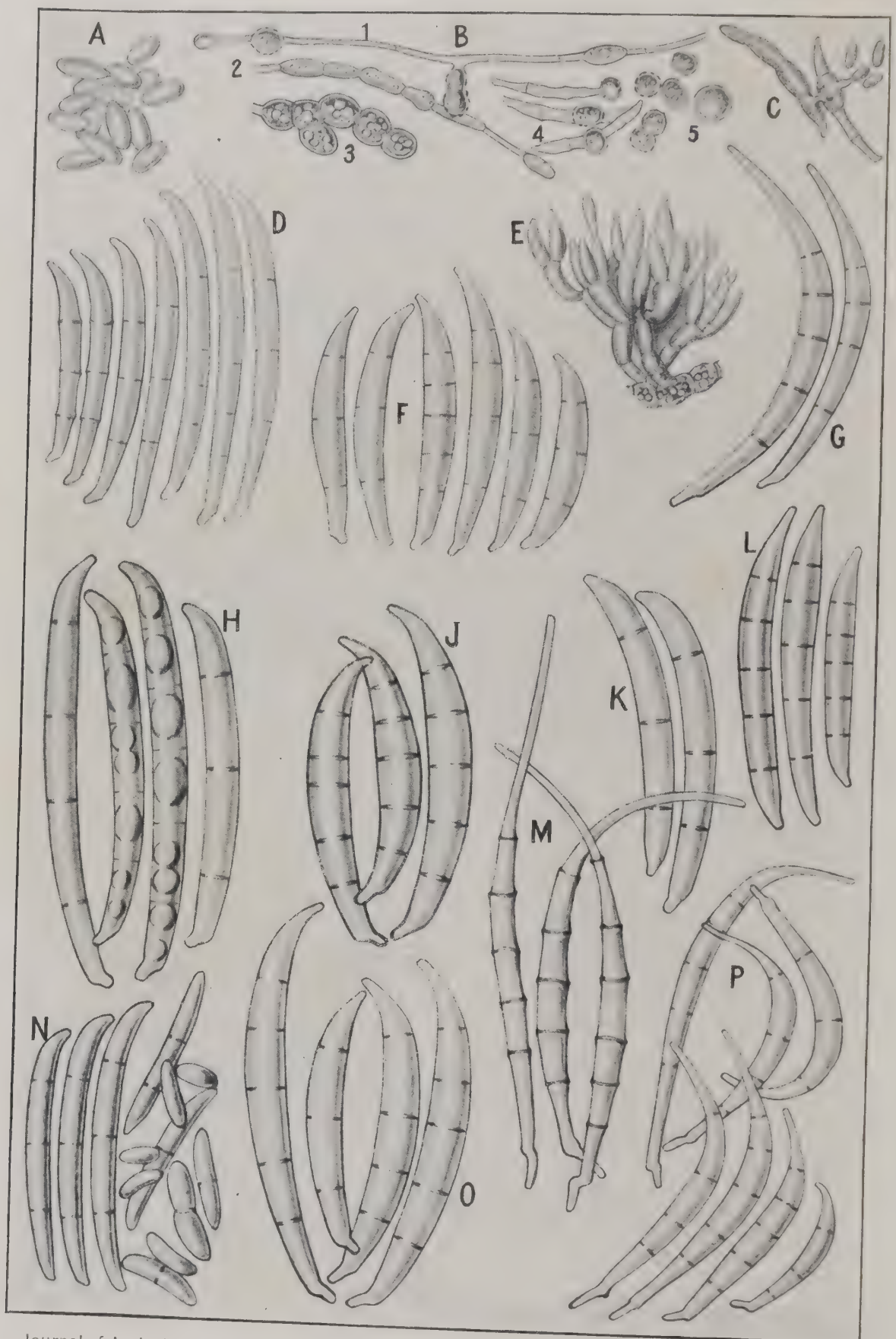
Fig. O.—*Gibberella Saubinetii* (Mont.) Sacc.

Fig. P.—*Fusarium caudatum* var. *volutum*, n. var.


A
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# MUTATION IN EGYPTIAN COTTON

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## INTRODUCTION

The occurrence of saltatory or discontinuous variations has frequently been reported by breeders of plants. Most of the instances mentioned in the older literature were not supported by unimpeachable evidence, but in the aggregate they established a strong presumption of the reality of this type of variation. Recent investigation has led to a better understanding of the phenomenon, which is now generally known as mutation.[1]

The publication of De Vries's work "Die Mutationstheorie" (1901–1903) [2] focused the attention of biologists upon the phenomenon of mutation by suggesting that it is not confined to domesticated organisms, but is also met with among wild species, and that organic evolution has taken place through the natural selection of mutations rather than of minor variations. While De Vries's theory of evolution has not won general acceptance, great interest attaches to his discovery of a plant in which mutation is a frequent rather than an extremely rare occurrence and from which numerous distinct and regularly heritable forms capable of description as elementary species or biotypes have been thus derived during a short period of time.

Among seed-propagated crop plants [3] there have been few well-substantiated instances of the origin by mutation of varieties which differ in several characters from the parental type, although their apparent rarity is perhaps attributable to imperfect knowledge of the history of

[1] Mutation in plants may be defined as a type of variation manifesting itself in the sudden appearance of a distinctly different individual the characters of which are uniformly expressed by its descendants when self-pollinated or cross-pollinated only among themselves.

This definition, which applies only to the higher plants, is purposely worded so as to exclude reference to the cause of mutation and to the conditions under which it takes place. Johannsen (1913, p. 161) has defined mutation as a sudden, discontinuous alteration of the biotype, independent of all crossing. This limitation of the term seems ill-advised, because it leaves us without a designation for the well-known cases which most biologists regard as the best examples of mutation and which represent a distinct and important phenomenon, although probably to be interpreted as resulting from remote or complex hybridization.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 301.

[3] "It must not be forgotten that the agricultural improved races do not possess the constancy of true species; whereas the varieties and subspecies of the horticulturist can only be distinguished from true species historically and systematically—not experimentally * * * In horticulture varieties arise by mutations, and varieties are elementary species. In agriculture, according to the current view and excepting in the instances of the unconscious isolation of elementary species, the highly improved races arise gradually through selection, but they never become species." (De Vries, 1909, v. 1, p. 82.)

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varieties in these groups.[1] Evidence is presented in this paper which is believed to justify the conclusion that mutation occurs in Egyptian cotton and that numerous varieties have thus arisen in Egypt and in Arizona.

Many biologists hold that mutation as observed by De Vries is an after-effect of hybridization. The mutability of Egyptian cotton is capable of a similar explanation, for it is widely believed that the type as a whole originated as a hybrid. It can at least be shown that the varieties now grown in Egypt, including the one which has given rise to the Arizona varieties, have been constantly exposed to crossing among themselves and with other types of cotton.

The breeder who works with a mutable group of plants has a great advantage in the ease with which new varieties can be fixed. It has been possible to maintain a high degree of uniformity in the varieties of Egyptian cotton which have arisen in Arizona by keeping each new form isolated from other types of cotton and by removing the relatively few aberrant plants from the seed increase fields before they come into blossom. In Egypt the maintenance of the cotton industry has largely depended upon the successive appearance of desirable mutants, since until very recently no adequate measures were taken to preserve a pure seed supply and each new variety rapidly deteriorated as a result of cross-pollination. While under the Arizona conditions deterioration is likely to be less rapid, the tendency of this type of cotton to produce an occasional valuable mutant may be regarded as a form of insurance against the possible "running out" of the present varieties.

The subjects treated in the following pages are: (1) The origin of Egyptian cotton, so far as it throws light upon the heterogeneous nature of this type and thus affords a possible explanation of its mutability; (2) the evidence for the mutational origin of the several varieties now grown commercially in Egypt; (3) the better known history of the Arizona varieties and the reasons for concluding that they have arisen by mutation, and (4) the evidence afforded by Egyptian cotton that mutability may be a result of hybridization.

The photographs used in illustrating this paper were made by Messrs. C. B. Doyle and Bruce Gilbert, of the Office of Acclimatization and Adaptation of Crop Plants and Cotton Breeding, Bureau of Plant Industry.

[1] There is some evidence that mutation occurs in tobacco. Mr. A. D. Shamel believes that the "Halliday" variety originated in this manner, although East and Hayes (1914) claim to have obtained an identical form in the $F_2$ of a Sumatra×Havana cross and consider it to represent merely a Mendelian recombination of the characters of the parent types. On the other hand, these authors (1914, p. 45) state that in tobacco "mutations may occur. We have shown the origin of one family by a very wide mutation. In this particular case it was not difficult to show that a constitutional change took place in a single germ cell of the mother plant."

A presumable case of mutation in barley has recently been described by L. Kiessling (1912).

## ORIGIN OF THE EGYPTIAN VARIETIES

Although less than 100 years have elapsed since Egyptian cotton was first recognized as a distinct type, there is much uncertainty about its origin. It is known that two or more species of Gossypium were cultivated in Egypt early in the nineteenth century. One of these, *G. vitifolium* Lam. (?), was a brown-linted tree cotton which resembled the Peruvian type. Another was the American Sea Island cotton (*G. barbadense* L.).

According to Prof. Balls (1912, p. 3–4) these species soon hybridized. Among the resulting recombinations was the low growing, brown-linted Ashmuni variety. The Mit Afifi was selected out of the latter in 1887, "and from this now degenerate complex of sub-varieties and splitting-forms other varieties have been selected." This view of the origin of the type is sustained by Mr. Frederic Fletcher (1908, p. 382), who states: "We have then in Delile's plant, *G. vitifolium* Lam. (and Cav.), the parent that mated with Sea Island cotton to form our present crop."[1]

The evidence seems conclusive that more than one species of Gossypium has contributed to the formation of the Egyptian type of cotton as we know it to-day. There can, at least, be no reasonable doubt that since the beginning of commercial cotton growing in Egypt the conditions have been and still are favorable to interspecific hybridization. Sea Island and American Upland cottons have been introduced from time to time, and the botanically very distinct "Hindi" cotton (referred doubtfully by Watt to *Gossypium punctatum* Sch. and Thon.) is almost everywhere present[2] and hybridizes with the Egyptian plants. Until very recently the importance of preventing crossing has been quite unappreciated in Egypt, and the only remedy for the rapid deterioration of the varieties has been the development of new ones.

The appearance about the year 1850 of the Ashmuni variety marks the close of the first period in the evolution of the Egyptian type of cotton. This brown-linted cotton was quickly recognized as representing a new commercial type, quite distinct from any cotton previously known in the markets of the world. Although formerly grown in the Nile Delta, the Ashmuni variety is now confined to the region south of Cairo. Its place in lower Egypt has been taken by the Mit Afifi and by other varieties derived from the latter. According to Balls (1912, p. 106) "Afifi was introduced commercially about 1887, Abbassi in 1893, Yannovitch in 1899, Nubari in 1907, Sakel in 1909, and Assili in 1910." Numerous other varieties have arisen from time to time, but either failed to attain much commercial importance or have been supplanted by other sorts.

[1] The extreme complexity of the botany of Egyptian cotton is apparent from the treatment of the subject by Watt (1907, p. 214, 223, 256, 292).

[2] Mr. O. F. Cook (1911) found Hindi plants in nearly every field which he inspected in Egypt, the proportion ranging from 1 to 20 per cent of the total number of plants.

While little is accurately known of the origin of most of these varieties, the evidence seems to Prof. Balls (1912, p. 105) to justify the conclusion that "the majority probably arose as single-plant 'selections.' In the case of Yannovitch this is definitely known."

The impression prevails in Egypt that these varieties can scarcely be distinguished from one another except in yield and lint percentage, in adaptability to particular soils and climatic conditions, and in the length, color, and fineness of the fiber. Nevertheless, when grown in Arizona from imported seed, most of them could readily be distinguished even in the blossoming period, or at the latest after the first bolls were set. In characterizing them, however, it was necessary to ignore the numerous hybrids with Hindi cotton which appeared in most of the plantings.

As compared with the Mit Afifi, the Ashmuni produced lower and more bushy plants. The Abassi variety had remarkably long and pointed, relatively slender bolls, very different from the plump, short-pointed bolls of the Mit Afifi (Pl. XXV, fig. 1). The Yannovitch plants averaged taller than the Mit Afifi, but there was so much variation among the plants grown from imported seed of this variety that a close comparison was impracticable. The Nubari variety differed from the Mit Afifi in its more compact habit of growth, in its larger, more frequently 5-lobed leaves, in having the bracts of the involucre (Pl. XXII, fig. 1) more grown together at the base, and in the much longer, more tapering bolls. The Sakellaridis differed from the Mit Afifi variety in many of its characters, notably in the much larger proportion of deeply 5-lobed leaves, involucral bracts with long teeth which extended nearly to the base of the bracts, and conical, very abruptly and very sharply pointed bolls (Pl. XXV, fig. 4). In the habit of growth, in the shape and small size of the leaves, and in the shape of the bolls the Sakellaridis variety showed some resemblance to Sea Island cotton.

The conclusion that these varieties originated by mutation is supported by the following facts: (1) The derivation of each from a single plant discovered in a field of very different cotton; (2) the distinctness of their botanical characters, especially in the recently developed Nubari and Sakellaridis varieties; and (3) their tendency to remain uniform, which is, however, finally nullified by the ample opportunities afforded in Egypt for cross-pollination with other types and for the mixing of seeds at the gins.

## ORIGIN OF NEW VARIETIES IN ARIZONA

Twelve years ago seed of the Mit Afifi variety, imported from Egypt, was planted at Yuma, Ariz. The resulting plants were generally unproductive, late in ripening, and produced fiber of poor quality. Selection carried on for several years resulted in some improvement in these respects, but the progress was not very encouraging. Although the plants showed considerable fluctuation, until 1908 there was no clear evidence that any of them had exceeded the limits of the characters of the Mit Afifi variety.

In that year two among the progeny rows were totally different in type from the parent variety and from one another. The characters of each were uniformly expressed in all plants of the row,[1] except the few and very different individuals which were obviously first-generation hybrids of Egyptian with Upland cotton. These two rows gave rise to the Yuma and Somerton varieties, described in an earlier publication (Kearney, 1910). Since both varieties appeared suddenly and were very uniform from the beginning when protected from cross-pollination with other types, the conclusion seems warranted that they were of mutational origin.

The Yuma variety was subjected during several years to yield tests and to mill tests, which showed the variety to be satisfactory in productiveness and in the spinning quality of the fiber. When a sufficient supply of pure seed had been obtained by carefully roguing the fields during three successive seasons, it was distributed to farmers in the Salt River Valley, Arizona, where this variety is now being grown on a commercial scale.[2]

Although the Somerton variety produced excellent fiber it was discarded because of its lateness in maturing and the excessive development of the vegetative branches.

Two other varieties, believed to be also of mutational origin, have since been developed in Arizona. They are here described under the names "Pima" and "Gila."

The contrasting characters of the Yuma, Pima, and Gila varieties are summarized in Table I.

TABLE I.—*Characters which distinguish the Yuma, Pima, and Gila varieties of Egyptian cotton*

| Character. | Variety. | | |
|---|---|---|---|
| | Yuma. | Pima. | Gila. |
| Vegetative branches. | Large, developing rapidly (Pl. XVIII, fig. 1). | Small, developing slowly or entirely wanting (Pl. XVIII, fig. 2). | Smaller than in the Yuma variety and developing less rapidly (Pl. XVIII, fig. 3). |
| Leaves of main stem. | A large proportion 5-lobed (Pl. XIX). | Usually deeply 5-lobed (Pl. XX). | Usually 3-lobed, when 5-lobed the basal lobes inconspicuous (Pl. XXI). |
| Involucres.... | Bracts usually much longer than wide, strongly connate (Pl. XXII, fig. 2). | Bracts not much longer than wide, separate or nearly so (Pl. XXIII, fig. 1). | Bracts not much longer than wide, separate or nearly so (Pl. XXIII, fig. 2). |
| Bolls..... | About twice as long as wide, tapering from near the base, not sharply pointed, deeply pitted (Pl. XXIV, fig. 2). | Nearly twice as long as wide, less tapering and more sharply pointed than in Yuma, shallow pitted (Pl. XXIV, fig. 3). | Considerably less than twice as long as wide, abruptly contracted at the blunt apex, deeply pitted (Pl. XXV, fig. 2). |
| Average length of fiber. | About 1½ inches..... | 1⅝ to 1¾ inches ..... | About $1\frac{7}{16}$ inches. |

[1] Some of the distinctive characters of each type were noted in the parent individual of the preceding year, but in neither case was it then recognized that a complete change of expression had taken place. Differences which seem very pronounced when expressed in the 50 or more plants of a progeny row may easily be overlooked in a single individual.

[2] The crop of 1913 amounted to about 2,100 bales, and about 15,000 acres were planted in 1914.

## THE YUMA VARIETY

As compared with the parent Mit Afifi, the Yuma variety is readily distinguished by its more frequently 5-lobed leaves (Pl. XIX); larger involucral bracts (Pl. XXII, fig. 2), which tend to be oblong-ovate rather than triangular-ovate and are usually united near the base so as to form a closed cup around the base of the boll; much longer and more tapering bolls (Pl. XXIV, fig. 2); and longer, lighter colored fiber. The fiber averages about 1½ inches long and resembles in color that of the Egyptian Yannovitch. In the characters of the foliage, involucres, and bolls the Yuma variety shows a striking resemblance to the Egyptian Nubari (Pl. XXII, fig. 1, and Pl. XXIV, fig. 1) which had appeared in Egypt three or four years earlier, presumably also by mutation from the Mit Afifi.[1] The fiber of the two varieties is quite different, however, that of the Yuma being longer and lighter colored.

The Yuma variety showed from the beginning a high degree of uniformity. In 1909 a 4-acre field was grown near Yuma, Ariz., having been planted with seed from those plants in the progeny row of 1908 which were not individually selected. Every plant in this field was examined in June, when 2 per cent of the total number were removed because they showed signs of hybrid origin or were otherwise undesirable. A second census in July resulted in the removal of an additional 0.5 per cent of the plants. The fact that not more than 2.5 per cent of the plants in this field showed a noteworthy departure from the type indicates a strong predominance of self-pollination or else a high degree of prepotency, since the progeny row of 1908 was situated between two rows of plants of wholly different character and since in 1906 and 1907 the stock from which this mutant came had been exposed to cross-pollination by Upland varieties of cotton.[2]

In 1913 Messrs. G. B. Gilbert and M. W. Buster, of the Office of Acclimatization and Adaptation of Crop Plants and Cotton Breeding, Bureau of Plant Industry, examined all the plants in two fields of the Yuma variety at Mesa, Ariz. Although these fields aggregated about 50 acres in extent and contained several hundred thousand plants, only about one dozen individuals were discovered which gave clear evidence of contamination with Hindi or with Upland cotton.

## THE PIMA VARIETY

The Pima variety originated in 1910 with a single plant of marked individuality which was found growing in a field of the Yuma variety. During the three subsequent generations this type has shown a striking

[1] Since the Mit Afifi seed with which the breeding work was begun in Arizona was imported in 1901, two years before the appearance of the parent individual of the Nubari variety; and since the latter is too distinct from Mit Afifi to be overlooked in progeny rows in which every individual plant was closely inspected, the possibility of a direct descent of the Yuma from the Nubari seems definitely excluded.

[2] Careful examination of all the plants in the Egyptian cotton progeny rows in 1908 showed that 8.1 per cent of the total number were Egyptian × Upland hybrids. In the Yuma variety row 7 plants out of 162, or about 4 per cent of the total number of individuals, were hybrids.

degree of uniformity in its very distinct botanical characters. The principal characters by which it differs from the parent Yuma variety are enumerated in Table I, p. 291. The Pima variety bears a marked resemblance to the discarded Somerton variety in the shape of the leaves, involucres, and bolls, but is almost the antithesis of that variety in its branching habit and seed characters. The Somerton showed an extreme development of the vegetative branches (much more so even than the Yuma) and had nearly smooth seeds, while the seeds of the Pima variety are very fuzzy for an Egyptian type.

## HISTORY OF THE PIMA COTTON

In 1909 several selections, made in the original progeny row (No. 382) of the Yuma variety, were grown as progeny rows at Sacaton, Ariz., and of these No. 382–10, which was thoroughly typical of the variety, proved to be the best. Several acres were planted in 1910 with bulk seed from this row,[1] and numerous individual selections were made in this field. Of these, plant No. 382–10–0–14 was the progenitor of the Pima variety. This plant attracted particular attention because of its large and very sharp-pointed bolls. An excellent progeny row was grown in 1911 from the seed of this individual, and five individual selections were made in this row. These selections were characterized by the marked reduction of the vegetative branches and by the retention of fruiting branches exceptionally low on the main stem. The selection which produced the best progeny the year following (plant No. 382–10–0–14–5) was noted as having the first fruiting branch low on the main stem (at node 10), the limbs much reduced, and the bracts nearly distinct.

The five progeny rows grown from these selections at Sacaton in 1912, when observed in July, greatly resembled each other and presented a very distinct and uniform type. They were in strong contrast to all other groups of progenies in the breeding nursery by reason of the marked reduction of the vegetative branches, which were generally fewer and were uniformly much shorter than in the Yuma variety. In one of the rows the reduction amounted to practical suppression. Correlated with this there was a strong tendency to retain the fruiting branches at a lower node of the main stem and to retain more bolls on the lower fruiting branches than is usual in the Yuma variety.

Row No. 382–10–0–14–5 proved more uniformly productive and long fibered than the other four, although the development of the vegetative branches was somewhat greater than in one of the other rows.[2] Twenty individual selections were made in this row and a smaller number in three of the other progeny rows of this type. Fourteen of the selections in row

[1] This field was carefully rogued, and the resulting seed was planted for increase in 1911. This was the source of the seed used in commercial plantings in 1912, 1913, and 1914. Hence, the Yuma variety as now grown by farmers in the Salt River Valley is derived from Selection No. 382–10.

[2] The 1913 progenies from selections in row No. 382–10–0–14–5 showed, however, less development of limbs than did the progenies from the other rows.

No. 382–10–0–14–5 were grown in progeny rows in 1913, when the superiority of this group as compared with the related groups of progenies was incontestable.

In July, 1913, careful examination failed to reveal any noteworthy departure from the type of the variety among the approximately 1,000 individuals in the progeny rows of this group. Hence, in the third generation from the parent individual this type showed practical uniformity in the expression of its botanical characters.

### CHARACTERS OF THE PIMA COTTON

Main stem stout, its internodes rather long, its first fruiting branch usually borne at the ninth or tenth node; vegetative branches few, remaining much shorter than the main stem and developing late or, frequently, altogether wanting; fruiting branches long, becoming pendulous, having a very long first internode; leaves large and thick, those of the main stem usually deeply 5-lobed, involucral bracts triangular ovate, separate or nearly so to the base; bolls large, plump, conical, very sharply and rather abruptly pointed, light green in color and not deeply pitted; seeds large, having both ends and often a part of their faces covered with bright green fuzz; fiber long (1⅝ to 1¾ inches), in color very pale buff with a tinge of pink.

### THE GILA VARIETY

The Gila variety originated with a plant discovered in 1908 by Mr. E. W. Hudson[1] in a field planted with the same stock of acclimatized Mit Afifi cotton which gave rise to the Yuma and the Somerton varieties. The distinctness of the characters of the parent individual and the uniformity with which these characters have been expressed in its descendants justify the conclusion that this variety, also, is of mutational origin. The Gila variety is very distinct from the Yuma variety. It resembles the Mit Afifi as grown in Arizona from imported seed, in the characters of the leaves, involucres, and bolls, but differs in its smaller vegetative branches, better fruiting branches, earlier ripening, much greater productiveness, and much longer and lighter colored fiber. Two selections made by Mr. Hudson in 1910, one having larger bolls and the other longer fiber than the parent variety, probably represent mutations from the Gila.

### HISTORY OF THE GILA COTTON

In regard to the parent individual, Mr. Hudson states that it had much browner fiber and ripened considerably earlier than the surrounding plants. In appearance the original plant was rather dwarf and the leaves very deeply lobed, much more so than in the average plants in the field, which gave a strikingly open appearance to the plant.

[1] An account of this variety is here presented with the concurrence of Mr. Walter T. Swingle, Physiologist in Charge of the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry. This office administers the Cooperative Testing and Demonstration Gardens at Sacaton, Ariz., of which Mr. Hudson is superintendent, in cooperation with the Bureau of Indian Affairs, Department of the Interior.

In 1909 a progeny row of 16 plants was grown at Sacaton from seed of this selection and showed a remarkable degree of uniformity in the plants and the fiber. In 1910 a half-acre plat was planted with the seed from this progeny row. During subsequent years the plantings have been gradually increased until, in 1913, 200 acres were grown on the Pima and the Maricopa Indian Reservations in southern Arizona (Pl. XVII).

## CHARACTERS OF THE GILA COTTON

Main stem rather slender, with relatively short internodes, its first fruiting branch usually borne at the ninth or tenth node; vegetative branches slender, developing late, usually remaining shorter than the main stem; fruiting branches with rather short internodes and numerous bolls; leaves rather small, those of the main stem usually 3-lobed, the lobes deep; involucral bracts triangular ovate, separate or nearly so to the base; bolls short, plump, abruptly narrowed to the blunt apex; seeds large, having usually one-third to one-half of the surface covered with fuzz; fiber about $1\frac{7}{16}$ inches long, somewhat darker colored than in the Yuma and Pima varieties.

## THE MUTABILITY OF EGYPTIAN COTTON

Four distinct varieties of Egyptian cotton—Yuma, Somerton, Pima, and Gila—have arisen in the course of plant-breeding work in Arizona. Three of these were derived directly from the Mit Afifi, while the Pima variety is an offshoot from the Yuma. Each of these varieties originated with a plant which was very different from the parent stock, and the distinctive characters have continued to be expressed with a high degree of uniformity during several generations. These facts seem to warrant the conclusion that the varieties mentioned are derived from mutants comparable with those of *Oenothera Lamarckiana* as described by De Vries.[1] The abrupt and distinct change of expression of characters in the parent individuals places the phenomenon outside the range of mere fluctuation, while the uniformity with which the new[2] characters have been expressed in each subsequent generation makes it wholly unlikely that these forms are an immediate product of hybridization.

As to the varieties which have originated in Egypt, while their history is much less completely known than that of the Arizona varieties, the data at hand point strongly to the conclusion that they also have been derived from mutants.

[1] The presence each year in the field plantings of the new varieties of Egyptian cotton of a small percentage of "off-type" plants is readily explained by the fact that the individuals which gave rise to the varieties were not protected from cross-pollination by surrounding plants of different character. De Vries (1909, v. 1, p. 275) states that before he resorted to bagging and self-pollinating the flowers of his mutants "these strains exhibited a very high degree of, though not an absolute, constancy."

[2] It is highly improbable that any of the characters exhibited by these mutants are new in the sense of having been absent in all lines of the ancestry. The history of Egyptian cotton indicates that more than one species of Gossypium has contributed to the formation of the type. It is therefore probable that a large share of the characters which are possessed by the different members of this genus have been transmitted in the Egyptian complex and may come to expression in its mutants.

A point of interest in connection with the origin of varieties in this type of cotton is the independent appearance of forms which are nearly identical in several of the characters by which they differ from the parent stock. As was pointed out on a preceding page, the Yuma variety is almost the exact counterpart, in foliage, involucres, and bolls, of the Nubari variety, which appeared in Egypt three years before the Yuma variety appeared in Arizona. Both varieties are derived from the Mit Afifi, which has very different characters. It has also been shown that the Pima variety, a descendant of the Yuma, bears a close resemblance in some of the characters by which it differs from its parent to the Somerton variety, which had appeared simultaneously with the Yuma three years previous to the appearance of the Pima variety. This independent appearance of the same or very similar new characters is paralleled in the case of *Oenothera Lamarckiana*, which has repeatedly given rise to identical or nearly identical mutants.

While mutation in these two plants appears to be essentially the same phenomenon, it is very much more active in *Oenothera Lamarckiana*. In De Vries's cultures during the first seven generations 1.5 per cent of the total number of individuals were mutants. On the other hand, in the Egyptian cotton grown in Arizona one conspicuously mutating individual among many thousand appears to be the limit of expectation. Even the heterogeneous stock of the Mit Afifi variety, which was introduced into Arizona 12 years ago, has produced only three or four mutants of a striking character, and in the more closely selected Yuma variety, which is now 7 years old, only one noteworthy mutant, the Pima, has been detected among the several thousand plants grown in progeny rows in each generation.[1]

The evidence at hand indicates that Egyptian cotton is a mutable group and that the mutability is of a type very similar to that occurring in *Oenothera Lamarckiana*. In seeking an explanation of the occurrence of mutation in Egyptian cotton it is therefore in order to consider certain theories which have been advanced to account for the mutability of Oenothera.

Shortly after the publication of De Vries's work it was suggested by Bateson and Saunders (1902) that the appearance of mutants in *Oenothera Lamarckiana* is due to hybridization. Other biologists have since adopted this idea. Thus, Tower (1910, p. 315–316), discussing the results of his experiments in crossing different species of Leptinotarsa, a genus of beetles in which, beginning with the sixth hybrid generation, the hybrid bred true except for the occasional appearance of mutant-like individuals, states: "These strains . . . gave results which strongly suggested that the interpretation of a mutative period, as described by De Vries in *Oenothera Lamarckiana*, may well be the variability which follows complex processes of hybridization."

[1] It does not follow that numerous minor or undesirable variants, eliminated each year in the process of roguing, might not, if subjected to the test of line breeding, prove to be mutants.

Davis (1911–1913) has sought to demonstrate experimentally that *Oenothera Lamarckiana*, which is not known to occur in the wild state anywhere in North America, originated as a hybrid between *O. biennis* and *O. grandiflora*.

Gates, while holding that *Oenothera Lamarckiana* could not have originated from a simple cross of *O. biennis* with *O. grandiflora*, believes that mutation and hybridization are associated phenomena. He says:

> Mutation in *O. Lamarckiana*, therefore, appears to be a condition of germinal instability and not a simple process of hybrid splitting, although this condition of instability has probably been brought about through previous crossing in the ancestry (Gates, 1911, p. 605; *see also* 1913a, p. 58–59).

On the other hand, Heribert-Nilsson (1912, p. 213) concluded from the results of his extensive hybridization experiments with this plant that the mutants can all be explained as either plus or minus combinations of characters already present in *O. Lamarckiana*. In his opinion instability of the germ plasm does not need to be assumed, and the whole phenomenon of mutation should be interpreted from one standpoint—that of Mendelian recombinations. He further concluded (1912, p. 218) that the mutants are not progressive or regressive new forms originated through the spontaneous appearance or disappearance of a single unit character—that is, through mutation in the sense of De Vries—but are minus combinations—that is, they have originated through the recombination of Mendelian characters already present in the parent species and distributed among different individuals.

In a recent paper, Gates (1913b) rejects these conclusions of Heribert-Nilsson on the ground that they are contrary to the cytological evidence and maintains the position (p. 298) that "mutation is an independent process requiring a special explanation."

The preponderance of evidence points to the conclusion that hybridization, possibly remote and of a complex nature, has been a factor in the mutability of Oenothera. On the other hand, the theory of Mendelian recombination does not afford adequate explanation of all the phenomena observed.

It remains to consider the evidence that hybridization has been a factor in the mutability of Egyptian cotton. The facts that hybrids between distinct types of cotton show great diversity in the $F_2$ and later generations and that it is difficult to obtain constant varieties by hybridization are well known to cotton breeders.[1] Yet, if the $F_2$ plants and their progeny during successive generations should be cross-

[1] The Foster variety is one of the few well-authenticated examples of a commercially important variety of known hybrid origin, having originated as the result of a cross between the Sunflower (a small-bolled long-staple Upland variety) and the Triumph (a large-bolled, short-staple Upland variety) made by Dr. D. A. Saunders. Although after several years of selection this variety has attained sufficient stability to warrant commercial production, the Foster variety is apparently much less uniform than the Yuma and other varieties of Egyptian cotton which have presumably originated by mutation. Individuals having the large, broad leaves and the short fiber of the Triumph variety are still frequently met with. (Cook, 1912, p. 17–18; 1913a, p. 16.)

pollinated with the same type which furnished one of the parents of the original hybrid, it is conceivable that finally only the characters of this parent would continue to be expressed. A hybrid of this kind, although very "dilute," might be expected to be in unstable equilibrium and, hence, to offer the proper conditions for the appearance of mutants.[1]

If we accept the hypothesis that mutability is a consequence of hybridization, it is not difficult to account for the tendency of Egyptian cotton to produce mutants. As was pointed out on preceding pages, the type as a whole is believed by some authorities to have originated from crosses between distinct species of Gossypium. But even if this theory be rejected, the possibility that hybridization has been a factor in the mutability of this cotton is not removed, since (1) the different varieties which, although genetically related, are distinct in their characters, are in Egypt often grown in close proximity and their seeds frequently become mixed at the gins, so that a great deal of crossing takes place among them; (2) other types of cotton which readily hybridize with the Egyptian, such as the American Upland and Sea Island, have been repeatedly introduced into Egypt and precautions to keep them isolated have rarely if ever been taken; (3) practically every cotton field in Egypt contains numerous plants of the very distinct Hindi type, which crosses with the Egyptian, producing more or less fertile offspring.[2]

The mixed condition of the principal Egyptian varieties has been strikingly exemplified in the Arizona plantings grown from imported seed. These always contain a greater or less number of Hindi plants and of first generation hybrids between these and the Egyptian, together with a multitude of individuals which give evidence of earlier crossing by their less pronounced expression of Hindi characters. Even the Sakellaridis variety, which is only about eight generations removed from the parent individual, has shown itself to be badly contaminated.

The theory that the mutability of Egyptian cotton is an after effect of hybridization with such distinctly different types as Hindi, Upland, or Sea Island, might be challenged on the ground that the recent mutants show no characters which can definitely be attributed to a non-Egyptian parent. This certainly appears to be the case with the Arizona varieties, which are purely Egyptian in the characters expressed. The objection may be met by assuming that the immediate ancestors of the mutating individuals were "diluted" hybrids, which, while expressing only Egyptian characters, were in a condition of unstable equilibrium, favorable to mutation. This assumption would imply that the remote non-Egyptian ancestor has made no direct contribution to the characters expressed in the mutating descendant and that the only remaining

[1] The possibility of inducing the production of desirable mutants by specially planned hybridization has been suggested by Mr. O. F. Cook (1913b, p. 86–87).

[2] According to Prof. Balls (1907, p. 57), "all varieties of Egyptian cotton, new or old, contain at least 50 per cent of plants with hybrid constitutions." In another work the same author (1912, p. 5) states: "The nominal varieties are more or less heterogeneous complexes of heterozygotes."

influence of hybridization is the disturbance of germinal equilibrium which manifests itself in the production of mutants. Since, furthermore, the distinctive characters of the mutants were not observed, singly or in combination, among nearly related individuals of the parent stock, the "germinal instability" theory of Gates appears preferable to the "Mendelian recombination" hypothesis of Heribert-Nilsson as an explanation of the known facts regarding mutation in Egyptian cotton.

Whatever may be the true explanation of the mutability of Egyptian cotton, there can be no question that the occurrence of mutants of a desirable character, and the relative uniformity during several generations of the resulting varieties, have been a safeguard to the cotton industry of Egypt. As fast as the old varieties have deteriorated through crossing with one another and with Hindi cotton, new varieties have been at hand to replace them.

In the varieties which have developed in Arizona, persistent roguing has so nearly eliminated the Hindi and Upland elements that they are scarcely detectable upon careful examination of thousands of plants (see p. 292). These varieties may therefore be expected to remain uniform much longer than those which are grown in Egypt, provided that they are kept isolated from each other and from other types of cotton. If, notwithstanding, deterioration should ultimately take place, the mutability of the type affords ground for the hope that new varieties of equal or greater value will be forthcoming. The contingency should, however, be borne in mind that these varieties, so long as they are protected from crossing with other forms, are likely to be less productive of mutants than was the heterogeneous stock of the Mit Afifi variety from which they originated.[1] It is significant in this connection that in the Yuma variety during seven generations only one striking and desirable mutant has been detected among thousands of plants and that in the Pima variety, which is derived from the mutant in question, no tendency to further mutation has yet been observed.

## SUMMARY

The origin of the Egyptian type of cotton is obscure. According to one theory, it is a product of hybridization between a brown-linted tree cotton and American Sea Island, both of these types having been cultivated in Egypt nearly a century ago. Whether or not this be true, there can be no question that the varieties now grown are of mixed ancestry, a condition which some investigators regard as favorable to mutation.

Numerous varieties have appeared from time to time in Egypt. The Ashmuni variety, now grown only in Upper Egypt, originated about 1850. This variety gave rise in 1887 to the Mit Afifi, and from the latter the

[1] "As a rule the new species proved much less mutable than the original *O. Lamarckiana* from which they originated. It is only the inconstant forms amongst them which exhibit a very high degree of mutability, as, for example, *O. scintillans*." (De Vries, 1909, v. 1, p. 296.)

Abassi, Yannovitch, Nubari, Sakellaridis, and Assil varieties have successively been developed.

As grown in Arizona from imported seed, most of the Egyptian varieties are readily distinguishable by the habit of the plants and by the characters of the leaves, involucres, and bolls, as well as of the fiber.

So far as the scanty evidence goes, each of these varieties originated with a mutant—i. e., an individual plant which showed an abrupt and definite change in the characters expressed. This conclusion is supported by the more complete data at hand regarding the history of the varieties which have been developed in Arizona.

Plant-breeding work in Arizona was begun 12 years ago with imported seed of the Mit Afifi variety. Persistent selection of the best plants caused some improvement in earliness and productiveness and in the quality of the fiber, but the progress was not very substantial prior to 1908, in which year two types very different from the Mit Afifi were recognized and isolated. One of these was the Yuma variety, now commercially grown in Arizona. This form has continued to express its distinctive characters with a high degree of uniformity, notwithstanding the fact that the parent individual and its immediate progeny were not protected against cross-pollination.

Two additional varieties, described in this paper under the names "Pima" and "Gila," have lately been developed in Arizona. The Pima variety appeared as a single plant of marked individuality in a field of Yuma cotton at Sacaton, Ariz., in 1910. Its characters have been expressed in its progeny with great uniformity during the three subsequent generations. This variety is easily distinguished from the parent Yuma variety by its relative limblessness and by the correlated retention of the lowest fruiting branches and bolls; by the more uniformly deeply 5-lobed leaves; by the shorter, relatively wider, and nearly separate involucral bracts; by the plumper and more abruptly and sharply pointed bolls; and by the longer fiber.

The Gila variety is derived from a single plant discovered by Mr. E. W. Hudson in a field of the acclimatized Mit Afifi stock grown at Sacaton, Ariz., in 1908. In its external characters this type resembles the parent Mit Afifi variety much more than the Yuma, but differs from the Mit Afifi in its earlier ripening, smaller vegetative branches, greater productiveness, and longer fiber. The individuality of the parent plant, together with the uniformity shown by its progeny during the subsequent generations, indicates that the Gila variety, like the Yuma and the Pima, is of mutational origin.

Egyptian cotton exhibits, although in a minor degree, the tendency to develop new varieties by mutation which characterizes *Oenothera Lamarckiana*. There is a further parallel in the fact that in both cases very similar, if not identical, new characters come into expression at

different times and in different places. An example of this phenomenon in Egyptian cotton is afforded by the Nubari and the Yuma varieties.

If the tendency to produce mutants is a result of remote or complex hybridization, the mutability of Egyptian cotton might be accounted for upon either of the following grounds: (1) The supposed hybrid origin of the type as a whole, or (2) later crossing with other types of cotton.

Ever since mutation became recognized as a factor in the breeding of Egyptian cotton the following methods have been followed in Arizona: (1) Recognition and isolation of desirable mutants; (2) selection and comparison on the progeny-row basis of those individuals among their progeny which express most fully the desirable characters of the new type; (3) elimination from the seed-increase fields, preferably before blossoming begins, of the aberrant and otherwise undesirable individuals.

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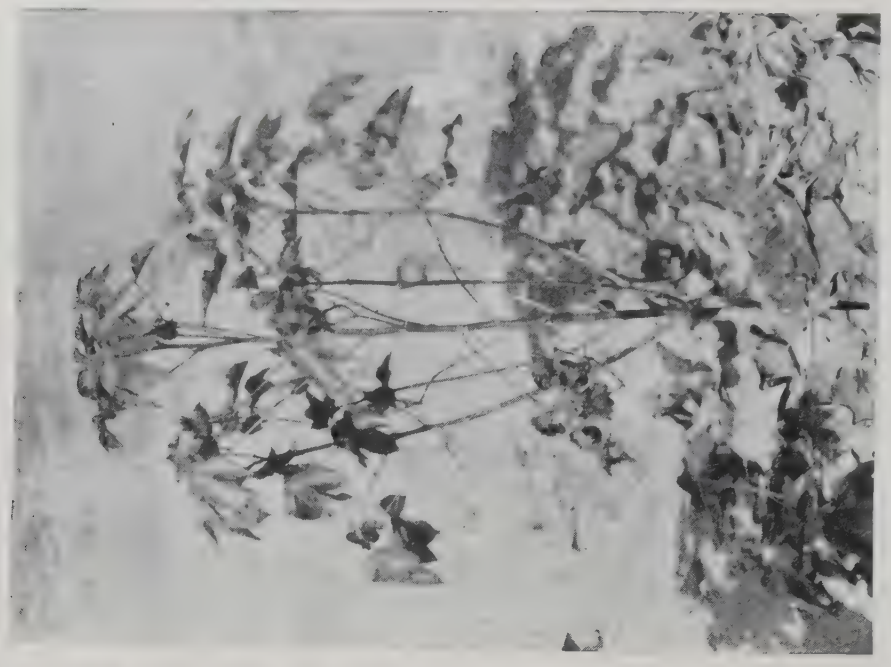
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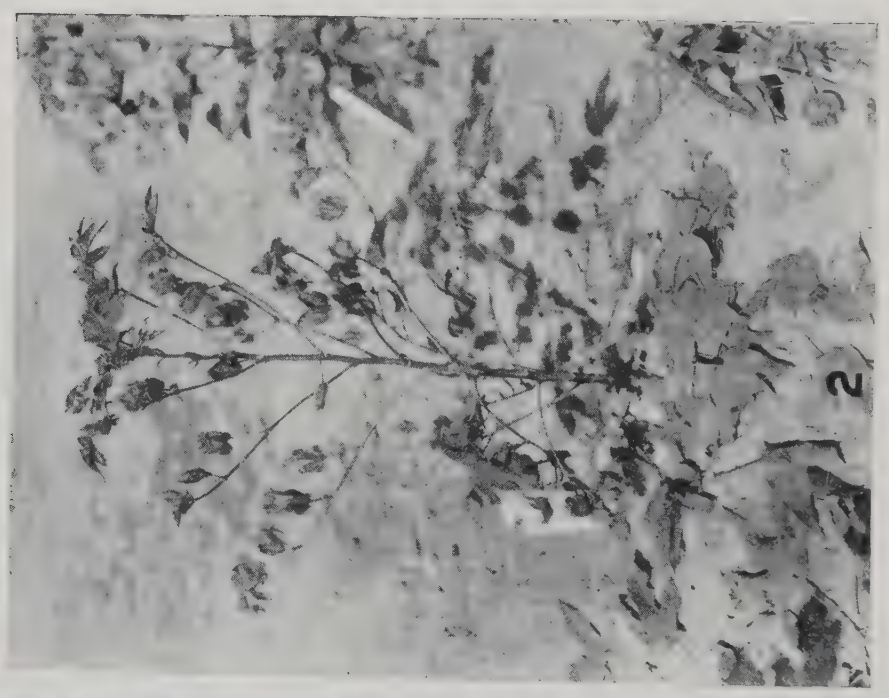
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## PLATE XVII

Field of Egyptian cotton (Gila variety) in blossom at the Cooperative Testing and Demonstration Gardens, Sacaton, Ariz., on July 15, 1913.




1


2


3

## PLATE XVIII

Fig. 1.—A plant of the Yuma variety of Egyptian cotton, photographed on July 15, 1913, showing the well-developed vegetative branches and the rather poorly developed lower fruiting branches.

Fig. 2.—A plant of the Pima variety of Egyptian cotton, photographed on July 15, 1913, showing the absence of vegetative branches and the presence of well-developed fruiting branches at the low nodes on the axis.

Fig. 3.—A plant of the Gila variety of Egyptian cotton, photographed on July 15, 1913, showing in comparison with the Yuma variety (Pl. XVIII, fig. 1) a smaller development of the vegetative branches.

PLATE XIX

Leaves of the Yuma variety of Egyptian cotton taken from the main stem and showing the strong tendency in this variety to produce 5-lobed leaves. (One-fourth natural size.)





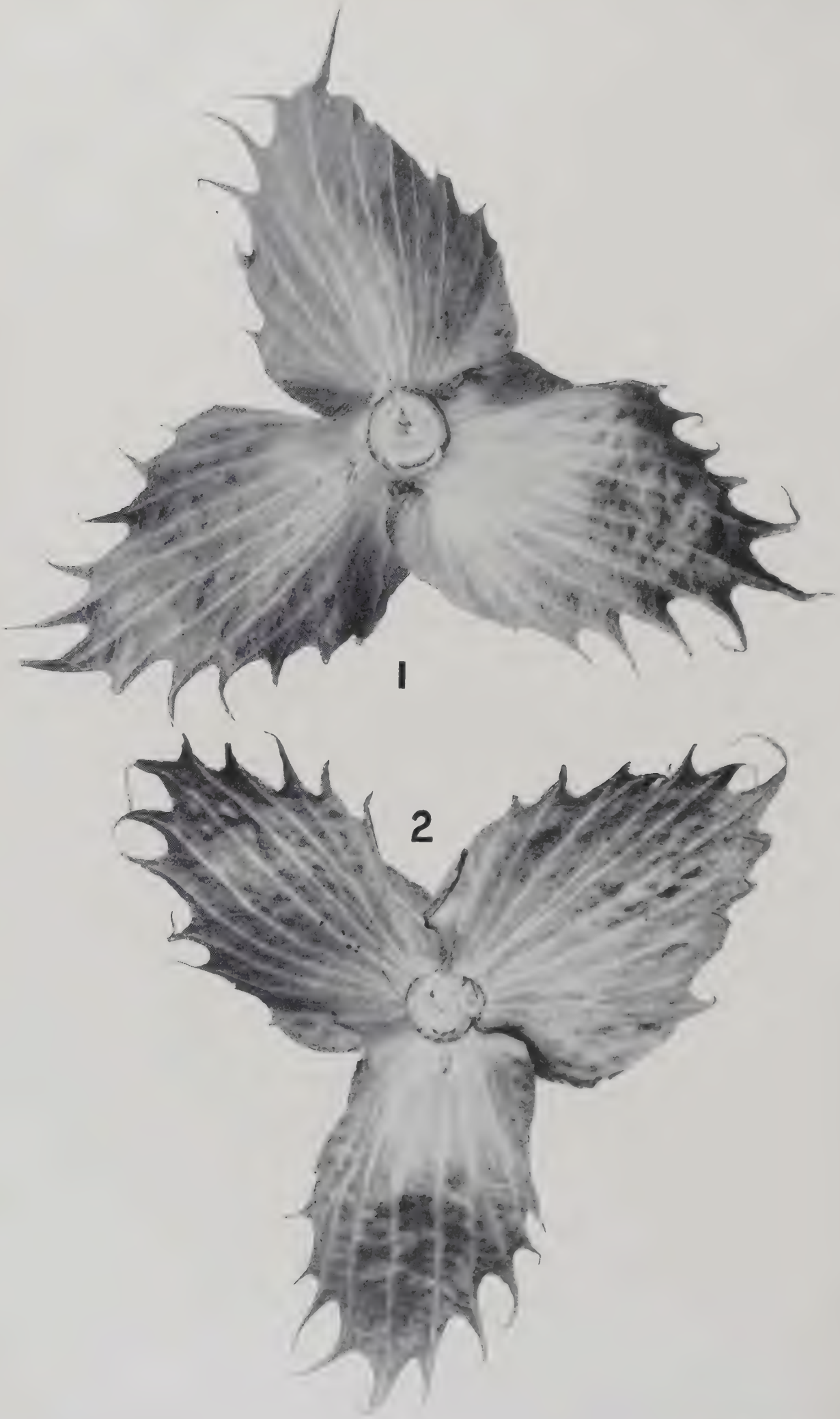
## PLATE XX

Leaves of the Pima variety of Egyptian cotton taken from the main stem. In this variety the leaves of the main stem are almost uniformly 5-lobed. (About one-third natural size.)

## PLATE XXI

Leaves of the Gila variety of Egyptian cotton taken from the main stem. In this variety the leaves are prevailingly 3-lobed, as is also the case in the Mit Afifi variety. (One-fourth natural size.)




1
2

## PLATE XXII

Involucres of Egyptian cotton (natural size).

Fig. 1.—Nubari variety.

Fig. 2.—Yuma variety.

In these two varieties the bracts are strongly connate, often forming a closed cup around the base of the boll.

## PLATE XXIII

Involucres of Egyptian cotton (natural size).

Fig. 1.—Pima variety.

Fig. 2.—Gila variety.

In these two varieties the bracts are shorter than in the Nubari and the Yuma varieties, and are separate, or nearly so, to the base. The Gila variety closely resembles the Mit Afifi variety in the character of the involucres.


1

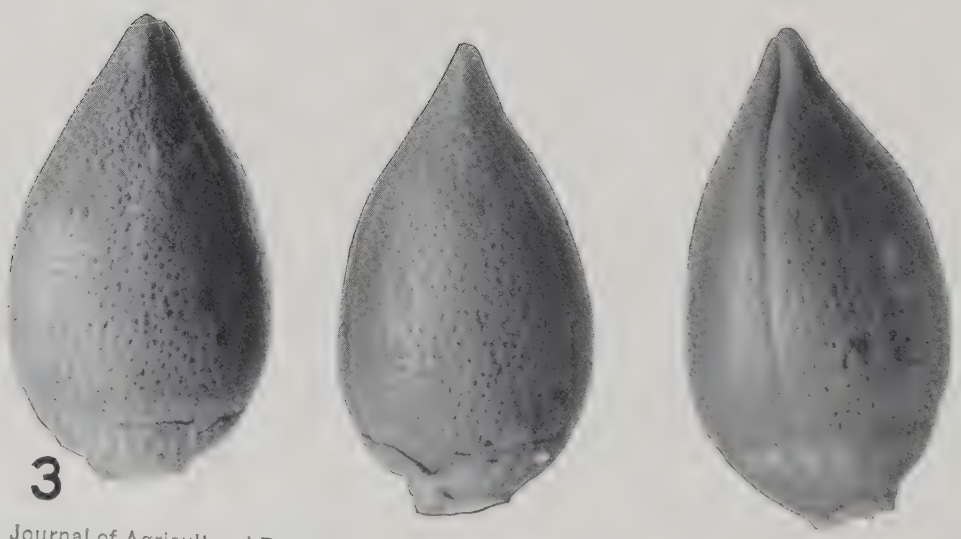

2



1



2



3

## PLATE XXIV

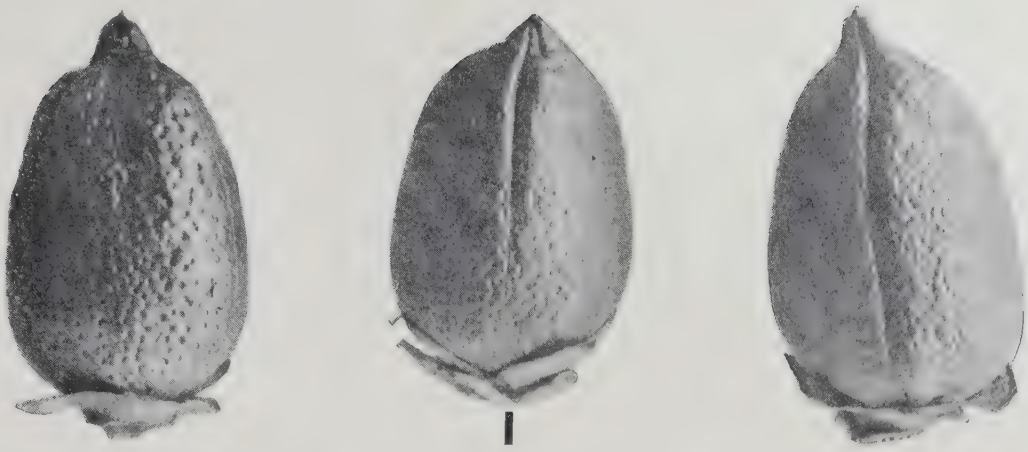
Bolls of Egyptian cotton (natural size).

Fig. 1.—Nubari variety.
Fig. 2.—Yuma variety.
Fig. 3.—Pima variety.
Note the close resemblance in the bolls of the Nubari and the Yuma varieties, and the different shape and shallower pitting of the bolls of the Pima variety.

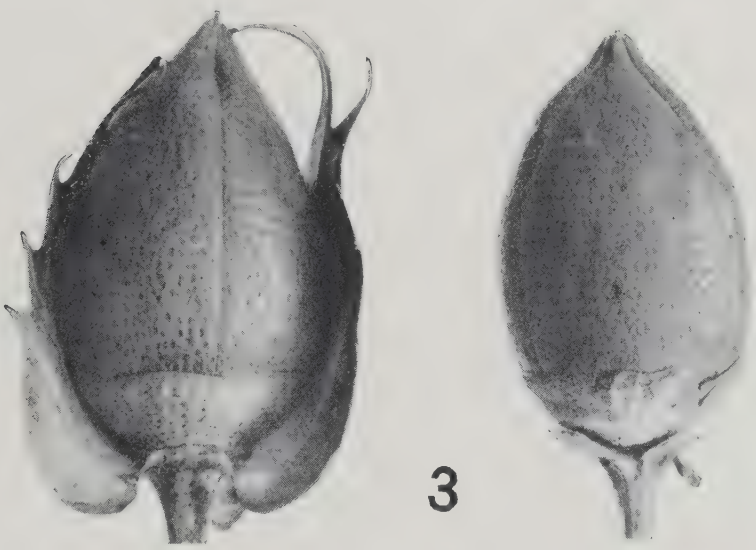
## PLATE XXV

Bolls of Egyptian cotton (natural size).

Fig. 1.—Mit Afifi variety.
Fig. 2.—Gila variety.
Fig. 3.—Big-bolled strain of the Gila variety.
Fig. 4.—Sakellaridis variety.


1


2


3


4

# INFLUENCE OF THE HOST ON THE MORPHOLOGICAL CHARACTERS OF PUCCINIA ELLISIANA AND PUCCINIA ANDROPOGONIS

By W. H. Long,

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Both *Puccinia ellisiana* Thuem. and *Puccinia andropogonis* Schw. have their telial stage on various species of Andropogon. For its æcial stage *Puccinia ellisiana* goes to certain species of Viola, while a number of species of Pentstemon are the æcial host for *Puccinia andropogonis*.

Two years ago the author [1] called attention to the fact that *Puccinia ellisiana* might have its æcial stage on Pentstemon as well as on certain species of Viola.

Culture data obtained in 1913 proved that this supposition was correct, as both Viola and Pentstemon were infected with *Puccinia ellisiana*. The changes produced in the morphological characters of the urediniospores when this rust was carried over to Pentstemon were so radical that the writer would not publish these culture data until they had been tested by another year's work. During the season of 1914, therefore, special attention was given to the infection of species of Viola and Pentstemon with *Puccinia ellisiana* and the infection of Pentstemon and Viola with *Puccinia andropogonis*. The culture work of 1914 agreed absolutely with the results obtained in 1913.

The remarkable feature of the results obtained was not the infection of two widely separated hosts by the same rust, but the changes produced in the morphology of the urediniospores of *Puccinia ellisiana* after passing through Pentstemon as the æcial host. The urediniospores of *Puccinia ellisiana* have thick verrucose walls, while those of *Puccinia andropogonis* have thin, echinulate walls. It is mainly on these well-marked and constant differences in the urediniospores that the two species are distinguished from each other. When urediniospores are not present in the teliosporic material the two rusts are often separated by determining by cultures which is the æcial host, Viola or Pentstemon, of the specimen in hand.

## CULTURE DATA FOR 1913

### DISSEMINATION OF PUCCINIA ELLISIANA AND PUCCINIA ANDROPOGONIS

The æciospores and urediniospores of both *Puccinia andropogonis* and *Puccinia ellisiana* are not carried far by wind currents. Six feet is the extreme distance yet observed by the writer for the spread of viable spores of either species to near-by stools of Andropogon.

[1] Long, W. H. Notes on three species of rusts on Andropogon. Phytopathology, v. 2, p. 164–171. August, 1912.

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Dept. of Agriculture, Washington, D. C.

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July 15, 1914
G–27

One patch of grass infected by *Puccinia andropogonis* has been observed by the writer for four years, and during this time the size of the infected area has not increased, though there are many stools of Andropogon near the infected area. In the center of this area are several infected groups of plants of *Pentstemon laevigatus*, while at a distance of 40 feet from these there are other plants of the same species. Yet during the last four years the rust has not crossed this 40-foot gap. Eight feet from the infected Pentstemon plants no signs of any rust on the grass can be found, even in the fall of the year after the rust has had all summer to spread. If the rust had ever crossed over the 40-foot gap to the grass near the uninfected Pentstemons, it certainly would have perpetuated itself, since both æcial and telial hosts were present and adjacent to each other. The rust had ample opportunity, as far as contiguity of telial host and proximity of infected plants is concerned, to be carried this distance. Nevertheless, it was limited to the stools of grass immediately adjacent to the infected æcial host, the Pentstemon.

Likewise the æciospores and urediniospores of *Puccinia ellisiana* are not carried over any great distance by the wind and can only infect stools of Andropogon which stand within about 6 feet of the æcial host. This has been previously noted by the writer.[1]

The difficulty with which the urediniospores of either *Puccinia andropogonis* or *Puccinia ellisiana* infect other stools of Andropogon was clearly shown when the writer attempted to obtain a large amount of teliosporic culture material by setting uninfected pots of Andropogon in actual contact with the pots containing the grass already infected from the æciospores. The experiment was a failure, as no infection occurred. The uninfected stools of grass remained free of the rust, even when the tips of the blades intermingled with the blades of the infected stool. Check stools situated 10 to 20 feet from the infected stools and planted in the same cold frame showed no infection.

For two years the writer has attempted to transfer the rust from stool to stool by inoculating with the urediniospores under bell jars in the greenhouse, but has been unsuccessful, even under such favorable conditions. This failure to infect with the urediniospores in the greenhouse may have been due to the high temperature and the condition of the blades of grass. It is probable that in nature these rusts are able to infect adjacent stools of grass on whose leaves the urediniospores fall, but to date the writer has been able to obtain infections on Andropogon only by means of the æciospores. These facts are given in detail to show the improbability of either of these two rusts being mixed in any of the culture material used for the experiments performed in 1913 and 1914.

For five years the writer has been studying in field and laboratory this group of Andropogon rusts, and during that time the peculiarities of

[1] Long, W. H. Op. cit., p. 170.

each have been noted. It is, therefore, with much confidence that the writer sets forth the facts as to the limited spread of each of these rusts in the uredinial stage.

The same thing is true to even a greater extent with *Uromyces andropogonis*. Only one time has the writer been able even to carry this rust from its æcial stage back to the Andropogon, and then only one or two sori were produced.

A careful study of the fresh urediniospores of both *Puccinia ellisiana* and *Puccinia andropogonis* under the microscope seems to indicate that the epispore in both species is either slightly viscid or slightly gelatinous; and, if this be true, it would explain the inability of the urediniospores to spread the rust to distant stools of Andropogon. However, the writer is not at all certain that the epispore when fresh has this viscid character, but the fact is fully established that in this region neither rust will spread very far from its æcial host. In fact, the writer has yet to see a stool of infected grass which had certainly been originally infected by urediniospores. In every instance the infected stools found even in the fall of the year were sufficiently close to the æcial host for the æciospores to have been the only and sole infecting agents. In this respect these rusts differ markedly from the common grain rusts, *Puccinia rubigo-vera* and *Puccinia graminis*, which are able to spread over large areas from the urediniospores alone.

## CHARACTER AND SOURCE OF CULTURE MATERIAL

In the culture work of 1913 the teliosporic material used was not pedigreed material from inoculations made under control conditions in the greenhouse, but was material grown for three years under the writer's direct supervision on a plat of ground near his residence in Clarendon, Va. The æcial host each year was *Viola sagittata*, and the telial host was *Andropogon virginicus*. That there are no Pentstemon plants within a radius of a mile of this place was determined by a careful search each year for the last five seasons. The plat of ground used was a glade surrounded on all sides by woods consisting of oaks (*Quercus* spp.) and pines (*Pinus* spp.).

The culture work of 1913 seemed to indicate that the species of the æcial host might influence to some extent the ability of *Puccinia ellisiana* to infect Pentstemon. For instance, teliosporic material whose æcial host was known to be *Viola sagittata* infected Pentstemon, while teliosporic material with *V. papilionacea* as its æcial ancestral host did not. In 1914, however, telial material from either æcial host readily infected the Pentstemon plants. The failure in 1913 of the teliosporic material which had *V. papilionacea* as its æcial ancestral host to infect Pentstemon was probably due to two things:

(1) The first series of inoculations with the rusts from this æcial host was made too early. The Pentstemon plants used had not yet reached their susceptible period.

(2) The second series of tests came so late in the season that it was either too hot in the greenhouse to infect the Pentstemon or the telial material had lost its ability to infect this unusual host under the existing conditions.

The writer used two species of Pentstemon in the culture experiments for 1913 and 1914, *Pentstemon laevigatus* from Virginia and *Pentstemon tubiflorus* from Texas. Only the former was infected by either *Puccinia andropogonis* or *Puccinia ellisiana*. When the leaves of *Pentstemon laevigatus* are first formed, they are covered with many small deciduous hairs, which fall off as the leaf gets older and leave the upper surface of the leaf nearly smooth. The leaves are usually not susceptible to either rust until after these hairs have disappeared. When the leaves get very old they lose their susceptibility entirely.

There is also often found intermixed with the usual form a strain of *Pentstemon laevigatus*, which retains its hairiness and is at all times immune to the rust.

During the spring of 1913 the writer continued the experiments reported for 1912 [1] with *Puccinia ellisiana* and *Uromyces andropogonis* Tracy and also carried on culture experiments with *Puccinia andropogonis* and a Puccinia from Oklahoma on *Andropogon furcatus*, whose æcial host proved to be *Oxalis stricta*. Only the results obtained from *Puccinia ellisiana* and *Puccinia andropogonis* are given and discussed here.

To show clearly the pedigree of each lot of teliosporic culture material used in the experiments, the æcial ancestral host for each year is given in the second column of Tables I–IV under the heading "Æcial ancestral host."

The teliosporic material used in all the experiments here recorded was on *Andropogon virginicus*, and the inoculations were made under control conditions in the greenhouses of the Department of Agriculture at Washington, D. C.

TABLE I.—*Teliosporic culture data for Puccinia ellisiana and P. andropogonis*

PUCCINIA ELLISIANA

| Species inoculated. | Æcial ancestral host. | Date of inoculation. | Degree of infection. | Pycnia. | Æcia. |
|---|---|---|---|---|---|
| | | 1913. | | 1913. | 1913. |
| Viola tricolor (cultivated pansy). | Viola sagittata | Apr. 7 | Very vigorous | Apr. 15 | Apr. 21 |
| Viola sororia | Viola papilionacea | Mar. 20 | Good | Apr. 1 | Apr. 14 |
| Do | do | Mar. 24 | do | Apr. 7 | Do. |
| Do | do | Apr. 15 | Fair | Apr. 22 | Apr. 30 |
| Do | do | Mar. 20 | Only one sorus | Mar. 28 | Apr. 2 |
| Viola sagittata | do | Mar. 24 | Good | Apr. 2 | Apr. 14 |
| Do | do | Mar. 28 | do | Apr. 7 | Apr. 16 |
| Viola hirsutula | do | do | Fair | do | Apr. 15 |
| Viola pedata | do | Mar. 20 | No infection | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Mar. 28 | do | | |

[1] Long, W. H. Op. cit., p. 164.

TABLE I.—*Teliosporic culture data for Puccinia ellisiana and P. andropogonis*—Contd.

PUCCINIA ELLISIANA—continued

| Species inoculated. | Æcial ancestral host. | Date of inoculation. | Degree of infection. | Pycnia. | Æcia. |
|---|---|---|---|---|---|
| | | 1913. | | 1913. | 1913. |
| Viola canadensis | Viola sagittata | May 13 | No infection[1] | | |
| Viola emarginata | Viola papilionacea | Mar. 20 | Poor | Apr. 1 | Apr. 8 |
| Do | do | Mar. 24 | Fair | Apr. 3 | Apr. 13 |
| Viola palmata | Viola fimbriatula | May 8 | Very vigorous | May 20 | May 27 |
| Viola triloba | Viola papilionacea | Mar. 28 | do | Apr. 8 | Apr. 15 |
| Do | do | May 8 | No infection | | |
| Do | Viola sagittata | Apr. 15 | Good | Apr. 28 | May 1 |
| Do | do | May 7 | do | May 18 | May 20 |
| Viola primulifolia | Viola papilionacea | Mar. 20 | No infection[2] | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Mar. 28 | do | | |
| Do | Viola sagittata | Apr. 2 | do | | |
| Oxalis stricta | Viola papilionacea | Mar. 20 | do | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Mar. 28 | do | | |
| Do | Viola sagittata | Apr. 2 | do | | |
| Do | do | Apr. 8 | do | | |
| Pentstemon laevigatus | Viola papilionacea | Mar. 20 | do | | |
| Do | do | Mar. 28 | do | | |
| Do | do | Apr. 15 | do | | |
| Do | do | May 5 | do | | |
| Do | Viola sagittata | Apr. 2 | Very vigorous | Apr. 8 | Apr. 20 |
| Do | do | Apr. 6 | do | Apr. 12 | Apr. 21 |
| Do | do | Apr. 7 | do | Apr. 15 | Apr. 24 |
| Do | do | May 7 | No infection[2] | | |
| Do | do | May 8 | do | | |
| Do | Viola fimbriatula | do | do | | |
| Do | do | do | do | | |

PUCCINIA ANDROPOGONIS

| | | | | | |
|---|---|---|---|---|---|
| Pentstemon laevigatus | Pentstemon laevigatus. | Mar. 20 | No infection[3] | | |
| Do | do | Mar. 26 | Vigorous | Apr. 2 | Apr. 12 |
| Do | do | Mar. 28 | do | Apr. 4 | Apr. 14 |
| Do | do | Apr. 2 | No infection | | |
| Do | do | Apr. 15 | | Apr. 22 | May 1 |
| Viola sagittata | do | Mar. 20 | No infection | | |
| Do | do | Mar. 26 | do | | |
| Do | do | Mar. 28 | do | | |
| Do | do | May 5 | do | | |
| Viola triloba | do | Apr. 28 | do | | |
| Do | do | May 15 | do | | |
| Viola papilionacea | do | Mar. 20 | do | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Mar. 26 | do | | |
| Do | do | Mar. 28 | do | | |
| Do | do | Apr. 4 | do | | |
| Viola pedata | do | Mar. 20 | do | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Mar. 28 | do | | |
| Do | do | Apr. 2 | do | | |
| Viola sororia | do | Mar. 20 | do | | |
| Do | do | Apr. 2 | do | | |
| Viola emarginata | do | Mar. 20 | do | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Apr. 7 | do | | |
| Viola primulifolia | do | Mar. 24 | do | | |
| Do | do | Mar. 28 | do | | |
| Oxalis stricta | do | Mar. 20 | do | | |
| Do | do | Mar. 24 | do | | |
| Do | do | Mar. 28 | do | | |

[1] Plant too old and weather too hot. [2] Weather too hot. [3] Plants not yet susceptible.

## CULTURE DATA FOR ÆCIAL INOCULATIONS MADE IN 1913 ON GRAMINACEOUS HOSTS

All the inoculations here recorded were made with pedigreed material grown under control conditions in the greenhouses of the Department of Agriculture at Washington, D. C.

TABLE II.—*Æcial inoculations with Puccinia ellisiana and P. andropogonis*

PUCCINIA ELLISIANA

| Species inoculated. | Æcial ancestral host. | Date of inoculation. | Degree of infection. | Uredinia. |
|---|---|---|---|---|
| | | 1913. | | 1913. |
| Andropogon virginicus | Viola papilionacea, 1912... / Viola sagittata, 1913 | May 5 | Vigorous | May 22 |
| Do | Viola sagittata, 1912... / Viola sagittata, 1913 | May 7 | No infection. Grass was diseased with a systemic smut. | ..... |
| Do | Viola sagittata, 1912... / Pentstemon laevigatus, 1913 | May 2 | Vigorous | May 19 |
| Do | Viola sagittata, 1912... / Pentstemon laevigatus, 1913 | May 5 | ....do | May 22 |
| Zea mays (corn) | Viola papilionacea, 1912... / Viola papilionacea, 1913... | Apr. 21 | No infection, but distinct pallid spots developed. | ..... |
| Do | Viola papilionacea, 1912... / Viola triloba, 1913 | Apr. 28 | No infection | ..... |
| Do | Viola sagittata, 1912... / Viola tricolor, 1913 (cultivated pansy). | ...do | ....do | ..... |
| Do | Viola sagittata, 1912... / Viola sagittata, 1913 | May 5 | No infection, but permanent yellow spots developed where germ tubes entered. | ..... |
| Sorghum halepense (Johnson grass). | Viola sagittata, 1912... / Pentstemon laevigatus, 1913 | Apr. 29 | No infection | ..... |

PUCCINIA ANDROPOGONIS

| | | | | |
|---|---|---|---|---|
| Andropogon virginicus | Pentstemon laevigatus... | Apr. 19 | Vigorous | May 2 |
| | ....do | May 5 | ....do | May 17 |
| Zea mays (corn) | ....do | Apr. 15 | No sori appeared, but yellow spots appeared on blades where inoculated. | ..... |
| | ....do | Apr. 19 | | |
| | ....do | May 6 | | |
| | ....do | May 15 | | |
| Kafir corn | ....do | May 5 | No infection, but reddish spots appeared where blades were inoculated. | ..... |
| | ....do | May 7 | | |
| Amber-seeded sorghum | ....do | May 5 | No infection | ..... |
| | ....do | May 7 | | |
| Milo maize | ....do | May 5 | ....do | ..... |
| | ....do | May 7 | ....do | ..... |
| Sorghum halepense | ....do | May 5 | ....do | ..... |

## CULTURE DATA FOR 1914

### CHARACTER AND SOURCE OF CULTURE MATERIAL

For the culture work performed in 1914 teliosporic material of *Puccinia ellisiana* was used from six different sources and material of *Puccinia andropogonis* from two different sources.

The teliosporic material of *Puccinia ellisiana* was (1) pedigreed material inoculated under control conditions in the greenhouses of the Bureau of Plant Industry at Washington, D. C.; (2) pedigreed material inoculated under control conditions at Clarendon, Va.; (3) pedigreed material from special selected areas near Courtlands, Va.; (4) pedigreed material from near Vinson, Va.; (5) material from a region free from Pentstemon at Spruce, Va.; and (6) pedigreed material obtained in 1913 by inoculating Pentstemon with the teliospores of *Puccinia ellisiana* under control conditions in the greenhouses of the Bureau of Plant Industry at Washington, D. C., and then using these æciospores to infect Andro-

pogon plants. In this paper this pedigreed teliosporic material (No. 6) is designated "*Puccinia ellisiana* from Pentstemon," in order to distinguish it from the teliosporic material of the ordinary *Puccinia andropogonis*. The source of each individual lot of inoculating material used in 1914 is noted in each table in column 3.

All of the inoculations were made in the greenhouses of the Bureau of Plant Industry at Washington, D. C., and all of the hosts were kept in the greenhouses until the urediniospores were fully developed. The stools of infected Andropogon were then transferred to widely separated cold frames between the different greenhouses where the urediniospores and teliospores could develop normally and yet be entirely free from chance contamination.

If the infected grass is kept in the greenhouse all summer and fall, the rust fails to spread and finally dies, so that no telial material whatever is obtained. This disappearance of the rust from the infected leaves is due to several causes, the most important of which are slugs, high temperature, dry air, and lack of dew at night when under glass in the greenhouse.

TABLE III.—*Teliosporic culture data for Puccinia ellisiana, P. ellisiana from Pentstemon, and P. andropogonis*

PUCCINIA ELLISIANA

| Species inoculated. | Æcial ancestral host. | Source of inoculation material. | Date of inoculation. | Degree of infection. | Pycnia. | Æcia. |
|---|---|---|---|---|---|---|
| | | | 1914. | | 1914. | 1914. |
| Viola tricolor (cultivated pansy). | Viola sagittata.. | Pedigreed, Vinson. | Apr. 17 | Vigorous..... | Apr. 28 | May 6 |
| Viola tricolor..... | .....do..... | .....do..... | May 12 | Poor..... | May 26 | June 4 |
| Viola papilionacea. | .....do..... | .....do..... | Apr. 17 | Fair..... | Apr. 29 | May 6 |
| Viola palmata..... | .....do..... | .....do..... | May 12 | .....do..... | May 26 | June 4 |
| Pentstemon laevigatus. | .....do..... | .....do..... | Apr. 17 | No infection [1]... | ..... | ..... |
| Do..... | .....do..... | .....do..... | May 12 | Fair..... | May 25 | June 2 |
| Do..... | .....do..... | Spruce..... | Apr. 19 | Good..... | May 10 | May 20 |
| Viola tricolor (cultivated pansy). | .....do..... | Pedigreed, Clarendon. | Apr. 17 | .....do..... | Apr. 30 | May 7 |
| Do..... | .....do..... | .....do..... | Apr. 20 | .....do..... | Apr. 29 | May 15 |
| Do..... | .....do..... | .....do..... | Apr. 23 | .....do..... | May 1 | May 12 |
| Viola sagittata..... | .....do..... | .....do..... | Apr. 17 | Fair..... | Apr. 29 | May 7 |
| Viola papilionacea.. | .....do..... | .....do..... | Apr. 16 | No infection..... | ..... | ..... |
| Viola canadensis... | .....do..... | .....do..... | May 11 | .....do..... | ..... | ..... |
| Pentstemon laevigatus. | .....do..... | .....do..... | Apr. 16 | Vigorous..... | Apr. 27 | May 7 |
| Do..... | .....do..... | .....do..... | Apr. 17 | No infection..... | ..... | ..... |
| Do..... | .....do..... | .....do..... | Apr. 20 | Fair..... | May 3 | May 16 |
| Do..... | .....do..... | .....do..... | Apr. 23 | .....do..... | May 7 | May 23 |
| Do..... | .....do..... | .....do..... | May 11 | No infection..... | ..... | ..... |
| Do..... | .....do..... | .....do..... | May 16 | .....do..... | ..... | ..... |
| Pentstemon tubiflorus. | .....do..... | .....do..... | Apr. 16 | .....do..... | ..... | ..... |
| Do..... | .....do..... | .....do..... | Apr. 23 | .....do..... | ..... | ..... |
| Viola papilionacea. | Viola papilionacea. | Pedigreed, Courtlands. | Apr. 17 | Fair..... | Apr. 29 | May 10 |
| Do..... | .....do..... | .....do..... | Apr. 28 | No infection..... | ..... | ..... |
| Viola tricolor..... | .....do..... | .....do..... | Apr. 17 | Poor..... | May 1 | May 10 |
| Viola canadensis... | .....do..... | .....do..... | May 8 | No infection..... | ..... | ..... |
| Pentstemon laevigatus. | .....do..... | .....do..... | Apr. 17 | .....do..... | ..... | ..... |
| Do..... | .....do..... | .....do..... | Apr. 28 | Good..... | May 10 | May 20 |
| Do..... | .....do..... | .....do..... | May 5 | No infection..... | ..... | ..... |
| Do..... | .....do..... | .....do..... | May 8 | Good..... | May 17 | May 26 |

[1] Pentstemon too young.

TABLE III.—*Teliosporic culture data for Puccinia ellisiana, P. ellisiana from Pentstemon, and P. andropogonis*—Continued

PUCCINIA ELLISIANA—continued

| Species inoculated. | Æcial ancestral host. | Source of inoculation material. | Date of inoculation. | Degree of infection. | Pycnia. | Æcia. |
|---|---|---|---|---|---|---|
| | | | 1914. | | 1914. | 1914. |
| Viola tricolor | Viola sagittata | Pedigreed, greenhouse. | Apr. 16 | No infection | | |
| Do | do | do | Apr. 20 | Good | Apr. 29 | May 5 |
| Do | do | do | May 1 | Fair | May 11 | May 22 |
| Viola tricolor arvensis. | do | do | Apr. 23 | do | May 8 | May 16 |
| Viola sororia | do | do | Apr. 16 | do | Apr. 28 | May 10 |
| Pentstemon tubiflorus. | do | do | do | No infection | | |
| Pentstemon laevigatus. | do | do | do | do | | |
| Do | do | do | Apr. 19 | Very vigorous | May 10 | May 20 |
| Do | do | do | Apr. 20 | Vigorous | May 1 | May 15 |
| Do | do | do | Apr. 21 | do | May 5 | May 12 |
| Do | do | do | Apr. 20 | do | May 2 | May 18 |
| Do | do | do | Apr. 23 | do | May 5 | Do. |
| Do | do | do | do | do | May 2 | May 15 |
| Do | do | do | Apr. 28 | do | May 9 | May 20 |
| Do | do | do | do | do | May 14 | May 21 |
| Do | do | do | do | do | May 8 | May 18 |
| Do | do | do | do | do | May 11 | May 20 |
| Do | do | do | Apr. 30 | do | May 15 | May 25 |
| Do | do | do | May 1 | do | May 9 | May 22 |
| Do | do | do | do | do | May 10 | Do. |
| Do | do | do | do | do | May 8 | May 21 |
| Do | do | do | do | do | May 10 | May 20 |
| Do | do | do | do | do | May 11 | May 22 |
| Do | do | do | do | do | May 9 | May 20 |
| Do | do | do | May 2 | do | May 14 | May 22 |

PUCCINIA ELLISIANA FROM PENTSTEMON[1]

| | | | | | | |
|---|---|---|---|---|---|---|
| Pentstemon laevigatus. | Viola sagittata, 1912. Pentstemon laevigatus, 1913. | Pedigreed, greenhouse. | Apr. 20 | Good | May 5 | May 20 |
| Do | do | do | May 1 | do | May 8 | May 22 |
| Do | do | do | May 8 | No infection | | |
| Viola tricolor (cultivated pansy). | do | do | Apr. 16 | do | | |
| Do | do | do | Apr. 20 | Very sparse | May 1 | May 8 |
| Do | do | do | Apr. 23 | do | May 5 | May 14 |
| Do | do | do | Apr. 28 | Vigorous | May 8 | May 20 |
| Do | do | do | May 1 | Sparse | May 14 | May 30 |
| Do | do | do | May 8 | do | May 20 | May 28 |
| Do | do | do | do | do | May 23 | June 4 |
| Do | do | do | do | do | May 18 | June 2 |
| Viola palmata | do | do | do | Vigorous | do | June 1 |
| Viola sagittata | do | do | Apr. 20 | No infection | | |
| Viola papilionacea | do | do | Apr. 16 | do | | |
| Do | do | do | Apr. 23 | do | | |
| Do | do | do | May 1 | do | | |

PUCCINIA ANDROPOGONIS[2]

| | | | | | | |
|---|---|---|---|---|---|---|
| Pentstemon laevigatus. | Pentstemon laevigatus. | Queen | May 1 | Very vigorous | May 9 | May 22 |
| Do | do | do | May 8 | Fair | May 19 | May 25 |
| Do | do | do | do | No infection | | |
| Do | do | do | do | Fair | May 20 | May 27 |
| Do | do | do | do | do | May 19 | May 26 |
| Do | do | do | May 16 | Very vigorous | May 29 | June 3 |
| Do | do | do | May 18 | Fair | May 27 | June 4 |

[1] All of the teliosporic material used in this set of experiments was obtained by inoculating *Pentstemon laevigatus* with the teliospores of *Puccinia ellisiana* in the spring of 1913. The resulting æciospores from the Pentstemon leaves were then sown on *Andropogon virginicus*. All was done under control conditions in the greenhouse. The rust thus obtained on *Andropogon virginicus* is here called "Puccinia ellisiana from Pentstemon," although it does not differ materially from the ordinary *Puccinia andropogonis* whose æcial host is normally Pentstemon.

[2] The teliosporic material used in these experiments was on *Andropogon virginicus* and came from two sources: (1) Pedigreed greenhouse material inoculated and grown under control conditions at the greenhouses of the Department of Agriculture at Washington, D. C.; and (2) pedigreed material grown under observation for 3 years at Queen, Va.

TABLE III.—*Teliosporic culture data for Puccinia ellisiana, P. ellisiana from Pentstemon, and P. andropogonis*—Continued

PUCCINIA ANDROPOGONIS—continued

| Species inoculated. | Æcial ancestral host. | Source of inoculation material. | Date of inoculation. | Degree of infection. | Pycnia. | Æcia. |
|---|---|---|---|---|---|---|
| | | | 1914. | | 1914. | 1914. |
| Pentstemon tubiflorus. | Pentstemon laevigatus. | Queen | May 1 | No infection | | |
| Do | do | do | May 8 | do | | |
| Do | do | do | May 16 | do | | |
| Viola papilionacea | do | do | May 1 | Sparse | May 10 | May 22 |
| Do | do | do | do | No infection | | |
| Do | do | do | May 8 | Sparse | May 20 | May 30 |
| Do | do | do | May 16 | No infection | | |
| Viola palmata | do | do | May 8 | Sparse | May 20 | May 29 |
| Viola triloba | do | do | do | No infection | | |
| Do | do | do | May 16 | do | | |
| Viola hirsutula | do | do | May 8 | do | | |
| Viola sororia | do | do | May 16 | do | | |
| Viola fimbriatula | do | do | do | do | | |
| Viola sagittata | do | do | May 1 | do | | |
| Viola tricolor | do | do | May 8 | do | | |
| Do | do | do | May 16 | do | | |
| Pentstemon laevigatus. | do | Greenhouse | May 1 | Good | May 9 | May 22 |
| Viola papilionacea | do | do | do | No infection | | |
| Do | do | do | do | 2 sori | May 9 | May 22 |

## CULTURE DATA FOR ÆCIAL INOCULATIONS MADE IN 1914 ON GRAMINACEOUS HOSTS [1]

TABLE IV.—*Æcial inoculations with Puccinia ellisiana, P. ellisiana from Pentstemon, and P. andropogonis*

PUCCINIA ELLISIANA

| Species inoculated. | Æcial ancestral host. | Date of inoculation. | Degree of infection. | Uredinia. |
|---|---|---|---|---|
| | | 1914. | | 1914. |
| Andropogon virginicus | Viola sagittata, 1913<br>Viola sororia, 1914 | May 22 | Good | June 4 |
| Do | Viola sagittata, 1913<br>Viola sagittata, 1914 | May 25 | Leaves old, but infection good. | June 12 |
| Do | Viola sagittata, 1913<br>Viola sagittata, 1914 | May 8 | Vigorous | May 25 |
| Do | Viola sagittata, 1913<br>Viola tricolor (pansy), 1914 | do | Fair | Do. |
| Do | Viola sagittata, 1912<br>Viola sagittata, 1913<br>Pentstemon laevigatus, 1914 | May 21 | Vigorous | June 1 |
| Do | Viola sagittata, 1912<br>Viola sagittata, 1913<br>Pentstemon laevigatus, 1914 | May 22 | do | Do. |
| Do | Viola sagittata, 1912<br>Viola sagittata, 1913<br>Pentstemon laevigatus, 1914 | May 25 | do | June 3 |

PUCCINIA ELLISIANA FROM PENTSTEMON

| | | | | |
|---|---|---|---|---|
| Andropogon virginicus | Viola sagittata, 1912<br>Pentstemon laevigatus, 1913<br>Viola tricolor (pansy), 1914 | May 12 | Poor | May 22 |
| Do | Viola sagitatta, 1912<br>Pentstemon laevigatus, 1913<br>Pentstemon laevigatus, 1914 | May 22 | Vigorous | June 1 |
| Do | Viola sagittata, 1912<br>Pentstemon laevigatus, 1913<br>Viola tricolor (pansy), 1914 | May 25 | Fair | June 10 |
| Do | Viola sagittata, 1912<br>Pentstemon laevigatus, 1913<br>Viola palmata, 1914 | June 1 | do | June 12 |

[1] All the inoculations here recorded were made with pedigreed material grown under control conditions in the greenhouses of the Department of Agriculture, at Washington, D. C.

TABLE IV.—*Æcial inoculations with Puccinia ellisiana, P. ellisiana from Pentstemon, and P. andropogonis*—Continued

PUCCINIA ANDROPOGONIS

| Species inoculated. | Æcial ancestral host. | Date of inoculation. | Degree of infection. | Uredinia. |
|---|---|---|---|---|
| | | 1914. | | 1914. |
| Andropogon virginicus | Pentstemon laevigatus, 1912.. Pentstemon laevigatus, 1913.. Pentstemon laevigatus, 1914.. | May 22 | Very vigorous | May 30 |
| Do | Pentstemon laevigatus, 1912.. Pentstemon laevigatus, 1913.. Pentstemon laevigatus, 1914.. | June 1 | Vigorous | June 15 |
| Do | Pentstemon laevigatus, 1912.. Pentstemon laevigatus, 1913.. Viola papilionacea, 1914 | May 22 | Good | June 1 |
| Do | Pentstemon laevigatus, 1912.. Pentstemon laevigatus, 1913.. Viola papilionacea, 1914 | May 29 | .....do | June 12 |

## DISCUSSION OF DATA

These tables show that the cycle from a graminaceous host back to a graminaceous host was completed for each species of rust under discussion. They also show that *Puccinia ellisiana* was carried over to Pentstemon, then to Andropogon, then back to both Pentstemon and Viola, and that this was done with pure pedigreed material grown under control conditions at the greenhouses of the Department of Agriculture, Washington, D. C. The cultures made in 1913 with *Puccinia ellisiana* show that the teliosporic stage of this rust is able to infect both Viola (its usual host) and Pentstemon (the common host for *Puccinia andropogonis*). These results were duplicated with pedigreed greenhouse material in the culture experiments made during 1914. The material used in 1914, as previously noted, came from six different sources. The material from five of these sources was pedigreed, and from three was both pedigreed and grown under control conditions. Precaution was taken to prevent contamination of the culture material and cultures throughout all of the experiments made in 1913 and 1914. The culture teliosporic material used was carefully checked under the microscope to further insure its purity. In no case were any signs of contamination found. The characteristic urediniospores for each rust were never found in the culture material of the other.

The author fully realized that on the purity of his teliosporic culture material would depend the validity of his entire series of experiments; hence every effort was made to prevent contamination, and apparently these efforts met with complete success. It was to prevent any chance contamination vitiating the experiments that material was used from so many (six) different sources, as it was very improbable that pedigreed material from widely separated areas would all be contaminated. The teliosporic material of *Puccinia ellisiana* from each of the six sources infected the Pentstemon plants and also infected the various species of

Viola used in each set of inoculations. In many instances the infection of the species of Pentstemon was very abundant and vigorous. This was especially true when the leaves of the *Pentstemon* sp. were at their optimum period of susceptibility.

*Puccinia ellisiana* did not infect the species of Pentstemon as abundantly as it did certain highly susceptible species of Viola. Nevertheless the infection of *Pentstemon* sp. was so great in many cases that if the material had been contaminated with *Puccinia andropogonis* the thin-walled, echinulate urediniospores characteristic of this rust would easily have been found in the culture material. In not a single instance was there a failure to infect the Pentstemon plants with *Puccinia ellisiana* if the leaves had reached their susceptible period. Two to four pots of species of Pentstemon were used in every set of inoculations, and not only was every plant infected, but in many instances every leaf and even the stem was attacked. In some cases the leaves were so badly infected that they died before all of the æcia opened.

When either "*Puccinia ellisiana* from Pentstemon" or the ordinary *Puccinia andropogonis* was used, the inoculated species of Pentstemon were more vigorously and abundantly infected than when typical *Puccinia ellisiana* material was used. However, when the attempt to carry "*Puccinia ellisiana* from Pentstemon" back to Viola was made, the rust would and did go on to Viola, but with great difficulty.

The data given in the tables do not show this condition fully, for out of nearly 50 pots of *Viola* spp. inoculated only 8 plants were infected. On these 8 plants the infection was very meager. On each of 2 of these plants three leaves were infected, but on each of the other 6 only one leaf was infected and only one sorus to a leaf developed. This is also true of *Puccinia andropogonis*. It will infect certain species of Viola, as the culture table shows, but only very sparingly and then only under the most favorable conditions.

The teliosporic culture material of "*Puccinia ellisiana* from Pentstemon" must be very virile and used in large quantities under the most favorable culture conditions and on a large number of plants of Viola to obtain any infection whatever; and when infection does occur only an occasional leaf out of a large number develops a sorus, while the check plants of Pentstemon used with *Viola* spp. under the same bell jar or in the same inoculating chamber are literally covered with sori. This means that hundreds of viable sporidia were discharged on to the violets and that only an occasional one was able to establish a foothold in the tissues of the violet and finally produce æcia. With less virile teliosporic material and under less favorable culture conditions, inoculations made on species of Viola with either *Puccinia andropogonis* or with "*Puccinia ellisiana* from Pentstemon" would probably fail to infect a single plant. This is exactly what happened in the culture experiments of 1913. No

infection whatever was obtained from *Puccinia andropogonis* when its teliospores were sown on violets.

The *Pentstemon* sp. inoculated with *Puccinia ellisiana* were infected about one-half as heavily as the *Pentstemon* sp. inoculated with *Puccinia andropogonis*, the usual Pentstemon rust, but no failure to infect susceptible species of Pentstemons with either rust occurred in the experiments of 1914.

A test was made with *Puccinia ellisiana* in the open. A small quantity of pedigreed teliosporic material of this rust grown under control conditions at the greenhouse was fastened among the leaves of a bunch of species of Pentstemon growing in open cold frames. The resulting natural infection was exceedingly vigorous and abundant. There were 10 to 15 æcial sori on some of the larger leaves, while every leaf (30) which reached the susceptible period during the viability of the teliospores was infected. This proves conclusively that in nature the ordinary rust on Viola (*Puccinia ellisiana*) is able to infect Pentstemon under the conditions normally obtaining in the field, and that all that is necessary is to have Pentstemon plants intermixed with stools of Andropogon which are infected with *Puccinia ellisiana*.

## INCUBATION PERIODS

Table V shows a very variable incubation period for each rust. This variability was to be expected, as it is well known to those mycologists who have done much culture work with rusts that the incubation period varies materially with the environment and host. It increases in length of time as the temperature rises in the greenhouses, and finally infection may cease entirely with extreme heat. But after making due allowances for these factors some interesting facts are seen when each year's cultures are compared. For instance, the incubation period of *Puccinia ellisiana* on violets for 1913 ranged from 13 to 25 days, with an average time of 18 days, while the same rust on Pentstemon in 1913 ranged from 15 to 18 days, with an average of 17 days. This shows a much greater variation in the range of the æcial incubation stage of *Puccinia ellisiana* when on Viola than on Pentstemon, but nearly the same general average. The greater variation in range on species of Viola is probably due to the fact that several species of Viola were used in the inoculation experiments, while only one species of Pentstemon was infected, since the species of Viola used often seems to influence to a limited extent the incubation period. In the cultures of 1914, however, there is about the same amount of variation for *Puccinia ellisiana* on Pentstemon as on Viola. (See Table V.)

TABLE V.—*Incubation period of each rust*

| Species of rust. | Incubation of æcia. | | | | Incubation of uredinia. | | | |
|---|---|---|---|---|---|---|---|---|
| | Host inoculated. | Period. | | | Host inoculated. | Period. | | |
| | | Year. | Range. | Average. | | Year. | Range. | Average. |
| | | | *Days.* | *Days.* | | | *Days.* | *Days.* |
| Puccinia ellisiana. | Viola spp..... | 1912 | 15 to 24 | 20 | Andropogon.. | 1912 | 12 | 12 |
| | .....do..... | 1913 | 13 to 25 | 18 | .....do..... | 1913 | 17 | 17 |
| | .....do..... | 1914 | 15 to 25 | 21 | .....do..... | 1914 | 13 to 18 | 16 |
| Do..... | Pentstemon sp. | 1913 | 15 to 18 | 17 | .....do..... | 1913 | 17 | 17 |
| | .....do..... | 1914 | 18 to 31 | 23 | .....do..... | 1914 | 9 to 11 | 10 |
| Puccinia ellisiana from Pentstemon. | Viola spp..... | 1914 | 18 to 29 | 23 | .....do..... | 1914 | 10 to 16 | 13 |
| | Pentstemon sp. | 1914 | 14 to 21 | 18 | .....do..... | 1914 | 10 | 10 |
| Puccinia andropogonis. | Pentstemon sp. | 1913 | 16 to 17 | 17 | .....do..... | ..... | 12 to 13 | 12.5 |
| | .....do..... | 1914 | 17 to 21 | 19 | .....do..... | ..... | 8 to 14 | 11 |
| | Viola spp..... | 1913 | ..... | ..... | ..... | 1913 | ..... | ..... |
| | .....do..... | 1914 | 19 to 22 | 21 | Andropogon.. | 1914 | 10 to 14 | 12 |

When an attempt is made to carry the rust on *Viola* spp. (*Puccinia ellisiana*) back from Pentstemon to Viola, the incubation period is materially lengthened. This is shown in Table V under "*Puccinia ellisiana* from Pentstemon, *Viola* spp., 1914," where the incubation stage ranges from 18 to 29 days, with an average of 23 days, while the same rust on Pentstemon ranges from 14 to 21, with an average of 18 days. The same lengthening of the incubation period also occurs when *Puccinia andropogonis* is carried over to species of Viola. In this case the range is 19 to 22, and the average is 21 days. In general, the incubation period in the change from Viola to Pentstemon is more uniform than in the change from Pentstemon to Viola.

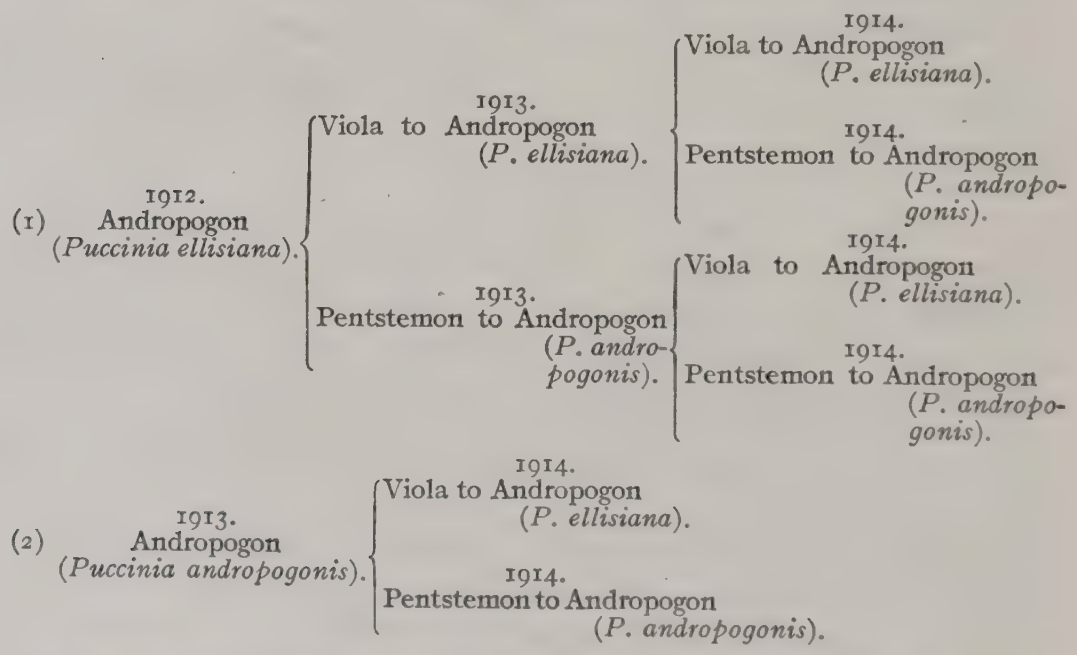
The short incubation period shown in Table V for the urediniospores for 1914 is due to the fact that the blades of Andropogon were very young and tender when inoculated. This condition of the grass was obtained by cutting all the first leaves off and forcing a new lot to develop in the greenhouse. On leaves which had developed in cold frames and then inoculated the incubation period was perceptibly longer.

A comparison of the averages of the incubation periods given in Table V for all the rusts on violets with those on Pentstemon shows that on the violet the general average is 21 days, while on Pentstemon it is 19 days. After making due allowance for the other factors known to influence the incubation period of rusts, this indicates that the æcial host and not the species of rust is the main factor in determining the variability and average length of the incubation period.

## MORPHOLOGICAL CHARACTERS

When *Puccinia ellisiana* infects Pentstemon and the resulting æciospores are sown on Andropogon, the urediniospores thus obtained are no longer typical *Puccinia ellisiana* spores, but have the thin echinulate walls characteristic of the ordinary Pentstemon rust, *Puccinia andropogonis*. When the teliosporic material thus obtained is sown on species of Viola and the æciospores from the infected violets are sown on Andropogon, the resulting urediniospores go back to the form typical of the regular Viola rust, *Puccinia ellisiana*. In other words, if the regular rust of Viola passes through Pentstemon, it develops urediniospores of the ordinary Pentstemon rust, *Puccinia andropogonis;* if it is now passed back through *Viola* spp., it develops the urediniospores of the typical Viola rust, *Puccinia ellisiana*. If, on the other hand, *Puccinia andropogonis* is sown on both Viola and Pentstemon, the *Viola* spp. will be very sparingly infected, as previously stated, and the æciospores from this infection when sown on Andropogon produce urediniospores typical of the regular Viola rust, *Puccinia ellisiana*, while if the same teliosporic material is sown on Pentstemon and the æciospores thus obtained are sown on Andropogon, urediniospores typical of the Pentstemon rust are developed. In each case the determining factor as to the characters of the urediniospores is the æcial host. This fact can be more clearly shown by the following diagram:

DIAGRAM SHOWING PLAN OF CROSS-INOCULATIONS WITH PUCCINIA ELLISIANA AND P. ANDROPOGONIS




All of the experiments represented in the above diagram were performed and all of the culture material used, both telial and æcial, was grown under control conditions in the Bureau of Plant Industry greenhouses at Washington, D. C. This diagram represents not only what

the author supposed might occur, but what actually did happen. The dates shown above each host indicate the year in which each set of experiments was performed.

In Table VI are grouped the principal morphological characters of each rust under discussion, so arranged that they can be readily compared.

TABLE VI.—*Morphological characters of Puccinia ellisiana and P. andropogonis*

| Stage of growth and morphology. | Puccinia ellisiana on or from Viola. | Puccinia ellisiana on or from Pentstemon. | Puccinia andropogonis on or from Pentstemon. |
|---|---|---|---|
| Æcia: | | | |
| Height | Variable, often very long (1 to 2 mm.). | Variable, usually very short (0.5 mm.). | Variable, very short (0.5 mm. or less). |
| Diameter | 275 to 410μ; average for 10, 350μ. | 235 to 315μ; average for 10, 260μ. | 225 to 315μ; average for 10, 250μ. |
| Æcial cavity | 110 to 165μ; average for 10, 145μ. | 115 to 150μ; average for 10, 130μ. | 125 to 165μ; average for 10, 135μ. |
| Peridia, color | Orange, slowly fading to white. | Pale yellow, quickly fading to white. | Pale yellow, quickly fading to white. |
| Peridia, segments. | Irregular, 4 to 10; not strongly reflexed. | Irregular, 3 to 6, strongly reflexed. | Irregular, 3 to 5, strongly reflexed. |
| Peridia | Opens tardily | Opens very soon | Opens very soon. |
| Æciospores: | | | |
| Shape | Subglobose | Subglobose | Subglobose. |
| Markings | Verruculose | Verruculose | Verruculose. |
| Size, range | 12 to 17 by 16 to 18μ | 16 to 20 by 18 to 23μ | 17 to 21 by 19 to 24μ. |
| Size, average | For 10 spores, 15 by 17μ | Average for 10 spores, 18 by 21μ. | Average for 10 spores, 19 by 22.5μ. |
| Urediniospores: | | | |
| Shape | Ellipsoid to subglobose | Subglobose to globose | Subglobose to globose. |
| Walls | Thick, 3 to 5μ; often thicker at apex. | Thin, 2μ, uniform | Thin, 1.5 to 2μ, uniform. |
| Markings | Verruculose; warts, 15 to 25 across spore. | Spinulose; spinules, 12 to 14 across spore. | Spinulose; spinules, 10 to 12 across spore. |
| Size, range | 16 to 19 by 21 to 23μ | 20 to 24 by 20.8 to 25.6μ | 21 to 23 by 22.4 to 25.6μ. |
| Size, average | Average for 10 spores, 17.5 by 19.5μ. | Average for 10 spores, 22.1 by 22.88μ. | Average for 10 spores, 22.3 by 23μ. |
| Germ pores | 4, equatorial | 4, equatorial | 4, equatorial. |
| Teliospores: | | | |
| Pedicel length | 16 to 64μ; average for 10 spores, 46μ. | 16 to 57μ; average for 10 spores, 39μ. | 16 to 45μ; average for 10 spores, 35μ. |
| Size, range | 16 to 23 by 32 to 45μ | 16 to 23 by 28 to 35μ | 16 to 24 by 32 to 40μ. |
| Size, average | Average for 10 spores, 20 by 38.5μ. | Average for 10 spores, 19.8 by 32.4μ. | Average for 10 spores, 20 by 35μ. |
| Apex | Thickened, 3 to 8μ | Thickened, 2 to 6μ | Thickened, 2 to 5μ. |

This table shows some very interesting things. For instance, under "Æcia, height," the characters of *Puccinia ellisiana* when on Pentstemon are practically identical with those of *Puccinia andropogonis;* under "Diameter" the characters are intermediate, but much nearer *Puccinia andropogonis* than *Puccinia ellisiana;* under "Peridia" a decided change is shown in color of peridia, number of segments, and time of opening, from the regular *Puccinia ellisiana* characters to those belonging to *Puccinia andropogonis.*

Under "Æciospores" the shape and markings of the spores of each rust are the same, but in size the æciospores of *Puccinia ellisiana* on Pentstemon are intermediate between the typical *Puccinia ellisiana* on Viola and *Puccinia andropogonis* on Pentstemon.

Under "Urediniospores" all of the fundamental differential characters of the urediniospores of *Puccinia ellisiana* (shape, size, apex, walls, and

markings on the walls of the spores) have been changed by the new æcial host, Pentstemon. The spores have changed from thick to thin walls, from verruculose to spinulose, from ellipsoid to globose, from 16 to 19 by 21 to 23$\mu$ to 20 to 24 by 20.8 to 25.6$\mu$, from walls with 15 to 25 warts across the spore to walls with 12 to 14 spinules across. In every instance the Viola rust has changed its characters to those of the ordinary Pentstemon rust. In the teliospores the same trend away from the characters of the typical Viola rust and toward those of the ordinary Pentstemon rust is seen. The characters of the teliospores of "*Puccinia ellisiana* from Pentstemon" are more nearly intermediate between the two regular rusts than are the characters of any of the other stages, but the differences in the characters of the teliospores of the typical *Puccinia ellisiana* and *Puccinia andropogonis* are so slight that one is usually not certain which rust he has unless the urediniospores are present. In other words the characters of each successive stage of *Puccinia ellisiana*, when it has Pentstemon for its æcial host, change to correspond to those of the ordinary Pentstemon rust, *Puccinia andropogonis*.

The large number of successful cultures made on Pentstemon with *Puccinia ellisiana*, the vigor and abundance of the infections obtained, the character of the culture material used, the many sources from which the culture material came, the use of pedigreed culture material grown under control conditions in the greenhouses, the special care taken in the actual culture work to avoid accidental contamination, and the duplication this year of last year's culture results, all prove conclusively that the results obtained in these experiments were not due to accidentally contaminated culture material—that is, to telial material containing viable spores of both rusts—but were due to changes produced by the æcial host through which the rust passed.

The infection of *Viola* spp. by *Puccinia andropogonis* and by "*Puccinia ellisiana* from Pentstemon" further corroborates the results obtained with *Puccinia ellisiana*. These experiments undoubtedly show that the ordinary Pentstemon rust, *Puccinia andropogonis*, can be produced from the Viola rust, *Puccinia ellisiana*, by simply passing Viola rust through Pentstemon as an æcial host. This process is so easy and the infection so vigorous and abundant that it certainly can and does occur in nature, thus originating the ordinary Pentstemon rust. But the reverse process, the passing of the regular Pentstemon rust through the *Viola* spp. and thus back to *Puccinia ellisiana*, is so difficult to accomplish even under the most favorable conditions that it seems probable that such a process would rarely, if ever, occur in nature.

*Puccinia ellisiana* and *Puccinia andropogonis* are then but different forms of the same species, since both can be produced from the same telial ancestor.

The modification of such profound morphological characters as shape, size, thickness, and markings of the walls of the spores by æcial or other hosts opens a broad and very important field for scientific research. It may prove to be the key to many anomalous conditions in the life history of the Uredinales which hitherto have appeared inexplicable.

The practical importance of these facts in relation to rusts of economic importance, such as those attacking cereals, truck crops, fruit and forest trees, is evident.

## SUMMARY

(1) *Puccinia ellisiana* has two widely separated æcial host genera, Viola and Pentstemon.

(2) The infection of Pentstemon by *Puccinia ellisiana* is vigorous and abundant.

(3) The characters of *Puccinia ellisiana* after passing through Pentstemon are entirely changed.

(4) The new characters assumed by *Puccinia ellisiana* correspond in every essential feature to those belonging to the Pentstemon rust, *Puccinia andropogonis*.

(5) The infection of *Viola* spp. by the ordinary Pentstemon rust, *Puccinia andropogonis*, also occurs.

(6) The characters of the rust obtained by inoculating species of Viola with *Puccinia andropogonis* are those of the regular Viola rust, *Puccinia ellisiana*.

(7) The transfer of *Puccinia ellisiana* from Pentstemon back to the Viola is much more difficult than that from the Viola to Pentstemon.

(8) *Puccinia andropogonis* may easily have originated in nature from *Puccinia ellisiana*.

(9) In the case of the rusts under consideration the determining factor as to the characters assumed by the spores is the æcial host.

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# ABILITY OF STREPTOCOCCI TO SURVIVE PASTEURIZATION

By S. HENRY AYERS, *Bacteriologist*, and WILLIAM T. JOHNSON, Jr., *Scientific Assistant, Dairy Division, Bureau of Animal Industry*

## INTRODUCTION

In this paper the group name "streptococcus" is used to designate bacteria which are spherical in form and which divide in one axis only, forming chains of from two to many cells. Among the pathogenic streptococci may be mentioned those causing inflammations and suppurations, of which *Streptococcus pyogenes* is an example. Among the nonpathogenic streptococci is a certain species of acid-forming bacterium which has been described as *Streptococcus lacticus*.

It is generally assumed that cocci do not form spores and the vegetative cells would not be expected to withstand Pasteurization. It has been shown, however, in previous publications,[1] that certain strains of lactic-acid bacteria, which would be classified among the streptococci, were able to survive Pasteurization. These strains had a high thermal death point; to destroy one culture in milk it was necessary to heat for 30 minutes at 75.6° C. (168° F.).

Pennington and Walter [2] also found that streptococci in cream survived Pasteurization, but they attributed this to the inefficiency of the Pasteurizing process.

It is evident that certain varieties of streptococci are able to survive Pasteurization, while other varieties are probably always destroyed.

Davis,[3] in a study of the streptococci in milk and their relation to septic sore throat, found that streptococci isolated from cases of sore throat were readily killed by heating at 60° C. (140 °F.) for 30 minutes.

Hamburger [4] found that a streptococcus isolated from a patient having septic sore throat was killed by heating to 62.8° C. (145° F.) for 20 minutes.

These results, together with the protection which proper Pasteurization seems to afford against epidemics of septic sore throat from milk supplies,

[1] Ayers, S. H., and Johnson, W. T., jr. The bacteriology of commercially Pasteurized and raw market milk. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 126, 98 p., 16 fig., 1910.

——— ——— A study of the bacteria which survive pasteurization. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 161, 66 p., 30 fig., 1910.

[2] Pennington, Mary E., and Walter, Georgiana. A bacteriological study of commercial ice cream. *In* N. Y. Med. Jour., v. 86, no. 22, p. 1013–1018, 1907.

[3] Davis, D. J. Bacteriologic study of streptococci in milk in relation to epidemic sore throat. *In* Jour. Amer. Med. Assoc., v. 58, no. 24, p. 1852–1854, 1912.

[4] Hamburger, L. P. The Baltimore epidemic of streptococcus or septic sore throat and its relation to a milk supply. *In* Bul. Johns Hopkins Hosp., v. 24, no. 263, p. 1–11, 8 fig., pl. 1, 1913.

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indicate that the varieties of streptococci associated with or responsible for this disease are among those varieties which have a low thermal death point.

## DETERMINATION OF THERMAL DEATH POINT

In these experiments the following method of determining the thermal death point has been used. The streptococci were grown first in plain neutral extract broth for 18 hours and then inoculated by means of a small-bore pipette into litmus-milk tubes. Four drops constituted an inoculation in each milk tube. In making the inoculation care was taken not to have any of the culture touch, or any of the inoculated milk wash up on, the sides of the tube, either during the handling or during the subsequent heating.

The inoculated milk tubes were heated in a large water bath and the temperature of the milk was recorded in a control milk tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary over half a degree in either direction. In all the experiments the heating period was 30 minutes at a given temperature. After heating, the tubes of milk were quickly cooled to about 10° C. (50° F.), incubated at 37° C. (98.6° F.), and the reactions recorded. Growth in the tube indicated that the organism was not destroyed at the particular temperature to which the milk had been subjected. In every case the tubes were run in duplicate, and in general both tubes had to show growth before the test was considered positive. The only exception to this was when only one tube showed growth after the highest heating temperature; in such cases one tube was considered a positive reaction, and the organism was recorded as surviving the process.

This method of determining the thermal death point was used in order to render the conditions of heating similar to Pasteurization.

### 1. THE THERMAL DEATH POINT OF THE CULTURES AS A WHOLE

The thermal death point of 139 cultures [1] of streptococci was studied. These cultures were isolated from cow feces, from the udder and mouth of the cow, and from milk and cream; therefore they represent a wide range of sources of the streptococci commonly found in milk.

The cultures were heated in milk, as previously described, to temperatures ranging from 48.9° C. (120° F.) to 73.9° C. (165° F.). The results given in Table I show the number and percentage of cultures which withstood the different temperatures. Of the total cultures, 138, or 99.28 per cent, survived heating for 30 minutes at 54.5° C. (130° F.). At 57.2° C. (135° F.) 118, or 84.89 per cent, of the cultures survived. At 60° C. (140° F.), the lowest Pasteurizing temperature used commer-

[1] The cultures of streptococci were supplied by Mr. L. A. Rogers, of the Dairy Division, Bureau of Animal Industry.

cially, 89, or 64.03 per cent, withstood the heating. When a temperature of 62.8° C. (145° F.) was used 46, or 33.07 per cent, of the streptococci survived. This temperature of 62.8° C. (145° F.) maintained for 30 minutes is the temperature generally used in the process of Pasteurization. At the higher temperatures the number of cultures which survived grew less as the temperature was increased. At 71.1° C. (160° F.) 3, or 2.58 per cent, of the streptococci survived, while at 73.9° C. (165° F.) all were destroyed.

These results are seen more clearly in figure 1, where they have been plotted. Some of the streptococci were destroyed at 54.5° C. (130° F.) and more at 57.2° C. (135° F.). It is particularly interesting to note that at 60° C. (140° F.) 89 of the cultures survived, while at 62.8° C.

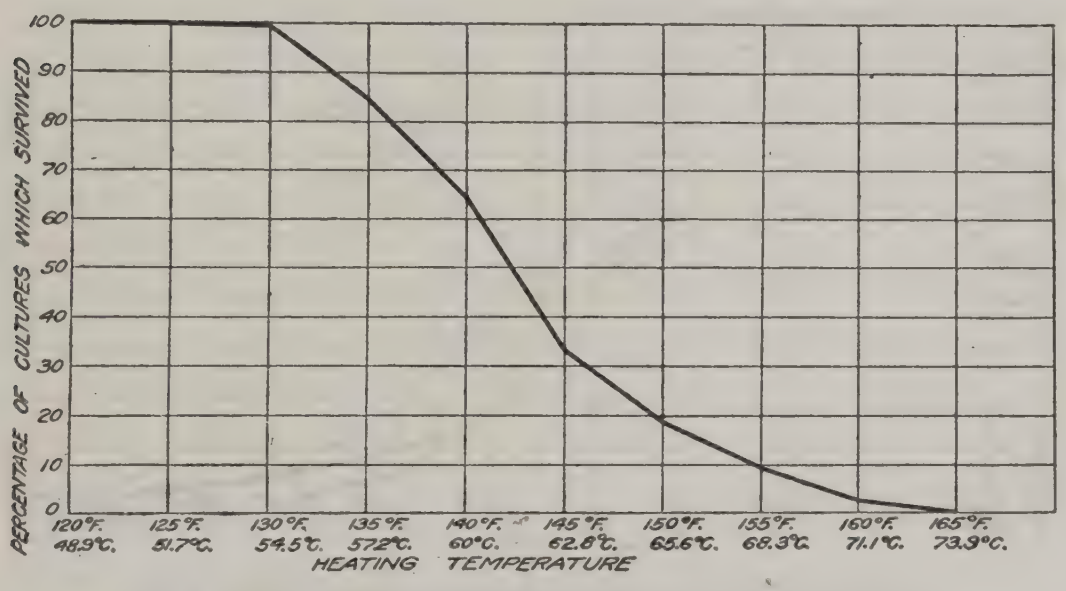



FIG. 1.—Results of heating streptococci for 30 minutes at various temperatures.

(145° F.), a difference of only 2.8° C., or 5° F., only 46 survived; therefore, 51.6 per cent of the streptococci which withstood 60° C. (140° F.) were destroyed at 62.8° C. (145° F.).

It is evident in the consideration of streptococci as a whole that a large percentage are able to survive Pasteurization.

TABLE I.—*Effect of heat on streptococci—all cultures*

| Item. | Cultures surviving after 30 minutes heating at— | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 48.9° C. (120° F.). | 51.7° C. (125° F.). | 54.5° C. (130° F.). | 57.2° C. (135° F.). | 60° C. (140° F.). | 62.8° C. (145° F.). | 65.6° C. (150° F.). | 68.3° C. (155° F.). | 71.1° C. (160° F.). | 73.9° C. (165° F.). |
| Number.. | 139 | 139 | 138 | 118 | 89 | 46 | 26 | 13 | 3 | 0 |
| Per cent.. | 100.00 | 100.00 | 99.28 | 84.89 | 64.03 | 33.07 | 18.71 | 9.35 | 2.58 | 0 |

## 2. THE THERMAL DEATH POINT OF THE CULTURES CLASSIFIED ACCORDING TO SOURCE

In order to determine whether streptococci from certain sources were more resistant to heating than others, the cultures have been grouped according to their sources. As before stated, the streptococci used in this study were isolated from cow feces, from the mouth and udder of the cow, and from milk and cream.

Table II shows that of the 45 cultures from cow feces, 44, or 97.77 per cent, survived 57.2° C. (135° F.). At 62.8° C. (140° F.) 31, or 68.88 per cent, survived. When heated to 62.8° C. (145° F.), 9 cultures, or 20 per cent, withstood the temperature. At 65.5° C. (150° F.) only

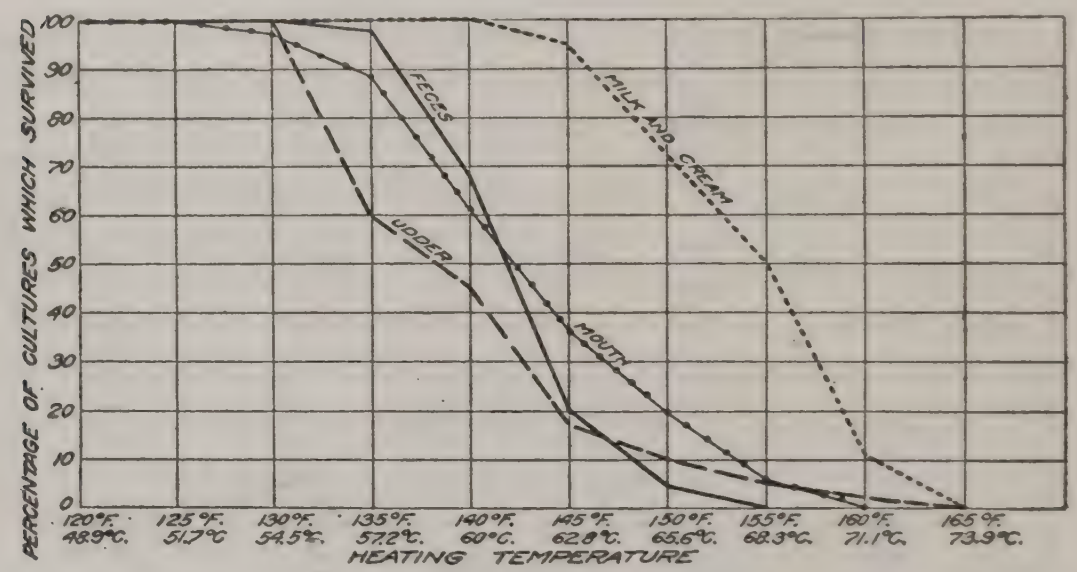



FIG. 2.—Results of heating streptococci (classified according to source) for 30 minutes at various temperatures.

4.44 per cent, survived, while at 68.3° C. (155° F.) all the streptococci were killed.

The cultures from the udder, as a whole, were less heat resistant than those from feces, although a few were able to withstand high temperatures. Of the 40 cultures 60 per cent were able to survive heating to 57.2° C. (135° F.). At 60° C. (140° F.) 45 per cent withstood the heating. At 62.8° C. (145° F.) 17.50 per cent survived. When heated to 71.1° C. (160° F.), 1 culture, or 2.5 per cent, still survived, but all were destroyed at 73.9° C. (165° F.).

In ability to withstand heat the streptococci from the mouth were very similar to those from feces.

The cultures isolated from milk and cream, however, were much more heat resistant than those from the three other sources. At 60° C. (140° F.) 18 cultures, or 100 per cent, survived. When heated at 62.8° C. (145° F.) 17, or 94.44 per cent, still survived. At 65.6° C. (150° F.) 72.22 per cent withstood the heating. Even at a temperature as high as

68.3° C. (155° F.) 9 cultures, or 50 per cent, survived. At 71.1° C. (160° F.) 2 cultures, or 11.11 per cent, survived, but all were destroyed at 73.9° C. (165° F.).

From these results, which are shown diagrammatically in figure 2, it is evident that the streptococci from the udder are, as a rule, less resistant to heat than those from the other sources. Those from the mouth and from the feces have about the same resistance, while streptococci from milk and cream were very much more heat resistant than those from other sources. See Table II.

TABLE II.—*Effect of heat on streptococci—cultures classified according to source*

| Source. | | Cultures surviving after heating for 30 minutes at— | | | | |
|---|---|---|---|---|---|---|
| | | 48.9° C. (120° F.). | 51.7° C. (125° F.). | 54.5° C. (130° F.). | 57.2° C. (135° F.). | 60° C. (140° F.). |
| Feces | Number | 45 | 45 | 45 | 44 | 31 |
| | Per cent | 100 | 100 | 100 | 97-77 | 68.88 |
| Udder | Number | 40 | 40 | 40 | 24 | 18 |
| | Per cent | 100 | 100 | 100 | 60 | 45 |
| Mouth | Number | 36 | 36 | 35 | 31 | 22 |
| | Per cent | 100 | 100 | 97.22 | 88.88 | 61.11 |
| Milk and cream | Number | 18 | 18 | 18 | 18 | 18 |
| | Per cent | 100 | 100 | 100 | 100 | 100 |

| Source. | | Cultures surviving after heating for 30 minutes at— | | | | |
|---|---|---|---|---|---|---|
| | | 62.8° C. (145° F.). | 65.6° C. (150° F.). | 68.3° C. (155° F.). | 71.1° C. (160° F.). | 73.9° C. (165° F.). |
| Feces | Number | 9 | 2 | 0 | 0 | 0 |
| | Per cent | 20 | 4.44 | 0 | 0 | 0 |
| Udder | Number | 7 | 4 | 2 | 1 | 0 |
| | Per cent | 17.50 | 10 | 5 | 2.50 | 0 |
| Mouth | Number | 13 | 7 | 2 | 0 | 0 |
| | Per cent | 36.11 | 19.44 | 5.55 | 0 | 0 |
| Milk and cream | Number | 17 | 13 | 9 | 2 | 0 |
| | Per cent | 94.44 | 72.22 | 50 | 11.11 | 0 |

### 3. THE THERMAL DEATH POINT OF THE TYPICAL AND ATYPICAL CULTURES

The writers do not consider the chain formation a proper basis on which to divide streptococci into typical and atypical groups. However, grouping is made on this basis in some board-of-health laboratories, and some investigators consider that chain-forming streptococci are associated with infected udders. For this reason the writers believe it of interest to consider the heat resistance of streptococci grouped as typical and atypical on the basis of chain formation.

Among the 139 cultures used in these experiments 22 formed long chains, and were for the purpose of this paper considered typical. The other 117 cultures formed chains of 10 or less, and were considered

atypical. Of the 22 typical streptococci 17 were from udders and 5 from cow feces.

The results in Table III show that the typical streptococci were less resistant to heat than were the atypical. Of the 22 typical cultures 12, or 54.54 per cent, survived 57.2° C. (135° F.). At 60° C. (140° F.) 9 cultures, or 40.91 per cent, withstood the heating. When heated to 62.8° C. (145° F.) 1 culture, or 4.54 per cent, survived, and all were destroyed at 65.6° C. (150° F.). This culture was isolated from cow feces. It is, of course, possible that if a larger number of cultures had been used some would have been found which would have withstood heating to higher temperatures.

Among the atypical cultures a much higher percentage were resistant to the higher temperatures. At 54.5° C. (130° F.) 99.14 per cent survived. When heated to 60° C. (140° F.) 68.37 per cent withstood the temperature. At 62.8° C. (145° F.) 38.46 per cent survived, and at 65.6° C. (150° F.) 22.22 per cent still survived. Even at 71.1° C. (160° F.) 3 cultures, or 2.56 per cent, withstood the heating. All were destroyed at 73.9° C. (165° F.). See Table III.

TABLE III.—*Effect of heat on streptococci—cultures classified as typical and atypical*

| Classification of cultures. | | Cultures surviving after heating for 30 minutes at— | | | | |
|---|---|---|---|---|---|---|
| | | 48.9° C. (120° F.). | 51.7° C. (125° F.). | 54.5° C. (130° F.). | 57.2° C. (135° F.). | 60° C. (140° F.). |
| Typical | Number | 22 | 22 | 22 | 12 | 9 |
| | Per cent | 100 | 100 | 100 | 54.54 | 40.91 |
| Atypical | Number | 117 | 117 | 116 | 106 | 80 |
| | Per cent | 100 | 100 | 99.41 | 90.59 | 68.37 |

| Classification of cultures. | | Cultures surviving after heating for 30 minutes at— | | | | |
|---|---|---|---|---|---|---|
| | | 62.8° C. (145° F.). | 65.6° C. (150° F.). | 68.3° C. (155° F.). | 71.1° C. (160° F.). | 73.9° C. (165° F.). |
| Typical | Number | 1 | 0 | 0 | 0 | 0 |
| | Per cent | 4.54 | 0 | 0 | 0 | 0 |
| Atypical | Number | 45 | 26 | 13 | 3 | 0 |
| | Per cent | 38.46 | 22.22 | 11.11 | 2.56 | 0 |

This marked difference in the heat resistance of typical and atypical streptococci is more clearly shown in figure 3.

Only 1 out of 22 cultures of the typical streptococci survived Pasteurization at 62.8° C. (145° F.) for 30 minutes. As shown in figure 3, the atypical cultures were much more resistant to heat.

From the results of these experiments it is evident that there is a considerable variation in the ability of streptococci to survive Pasteurization, and a general consideration of this ability seems of interest.

## RESULTS OBTAINED IN THE EXPERIMENTS

Two classes of streptococci are able to withstand Pasteurizing temperatures, and this is also true of other groups of nonspore-forming bacteria:

Class 1.—Those streptococci which may have a low majority thermal death point but a high absolute thermal death point.

Class 2.—Those streptococci which have a high majority thermal death point.

The terms "high majority thermal death point" and "low majority thermal death point," suggested by Gage and Stoughton,[1] mean the temperature at which the majority of the bacteria are destroyed. In class 1, therefore, the majority thermal death point of the streptococci might be below the Pasteurizing temperature, and they would therefore be destroyed. However, a few bacteria more resistant than the others

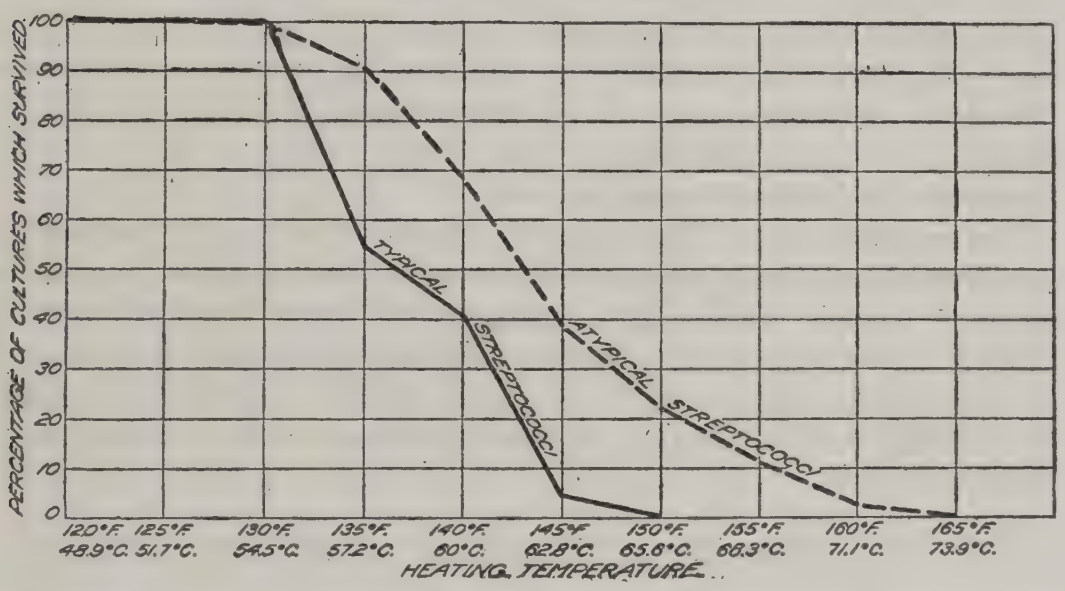



FIG. 3.—Results of heating streptococci (classified as typical and atypical) for 30 minutes at various temperatures.

might survive the Pasteurizing temperature and then continue to develop in the Pasteurized milk. The fact that some of the streptococci which were studied would fall in this class was plainly shown in the experiments of the writers. Often, after a tube of milk containing a culture of streptococci had been heated, the reaction indicating their growth would be shown in 24 hours, while in other cases five or six days' incubation was necessary in order to show an acid reaction, thus indicating growth. In such cases it was evident that only a few bacteria survived the heating. Among this class of streptococci it is quite impossible to say whether a few cells survive high temperatures because of certain resistant qualities peculiar to themselves or whether they are protected in some way in the milk in which they are heated.

[1] Gage, S. de M., and Stoughton, Grace Van E. A study of the laws governing the resistance of Bacillus coli to heat. *In* Technol. Quart., v. 19, no. 1, p. 41–54, 1906.

The second class of streptococci survive Pasteurization because of the fact that their majority thermal death point is above the Pasteurizing temperature. As previously stated in this paper, the writers isolated a lactic-acid streptococcus from Pasteurized milk which required 30 minutes' heating at 75.6° C. (168° F.) to destroy it. During the summer of 1909 some experiments were made to determine the effect of Pasteurization for 30 minutes on this culture at various temperatures. In February, 1914, after having kept this culture by repeated transfers in sterile milk, the experiment was again repeated. As may be seen from Table IV, in the experiments in 1909 there was no reduction of bacteria of this culture when Pasteurized for 30 minutes at 60° C. (140° F.) or 65.6° C. (150° F.), but there was a reduction of 53.07 per cent when heated at 71.1° C. (160° F.) for 30 minutes.

In the repeated experiment with the same culture, about 4½ years later, similar results were obtained. At 60° C. (140° F.) and 65.6° C. (150° F.) there was practically no reduction in the bacterial numbers. The slight difference noted is within the experimental error, as this culture grows with difficulty on solid media. At 71.1° C. (160° F.) there was a reduction of 99.20 per cent.

This organism has a high majority thermal death point, as may be seen from these results, and is able to survive Pasteurization because its majority thermal death point is above the temperature of 62.8° C. (145° F.), the temperature generally used in commercial Pasteurization with the holder process. It is also interesting to note that the ability to resist high temperatures is a permanent characteristic of this organism. (See Table IV.)

TABLE IV.—*The majority thermal death point of a lactic-acid-forming streptococcus*

EXPERIMENT DURING SUMMER OF 1909

| Temperature. | Number of bacteria per c. c. | | Percentage of reduction. |
|---|---|---|---|
| | Before heating. | After heating. | |
| 60° C. (140° F.) | 58,000,000 | 63,000,000 | 0 |
| | 64,500,000 | 51,000,000 | |
| | [a] 61,250,000 | [a] 57,250,000 | |
| 65.6° C. (150° F.) | 41,000,000 | 41,700,000 | 0 |
| 71.1° C. (160° F.) | 244,000,000 | 133,000,000 | ..... |
| | 315,000,000 | 138,000,000 | ..... |
| | [a] 284,500,000 | [a] 135,500,000 | 53.07 |

[a] Average.

TABLE IV.—*The majority thermal death point of a lactic-acid forming streptococcus*—Con.

EXPERIMENT DURING WINTER OF 1914

| Temperature. | Number of bacteria per c. c. | | Percentage of reduction. |
|---|---|---|---|
| | Before heating. | After heating. | |
| 60° C. (140° F.) | 1,400,000<br>1,600,000<br>[a] 1,500,000 | 1,040,000<br>1,180,000<br>[a] 1,110,000 | 0 |
| 65.6° C. (150° F.) | [a] 1,500,000 | 1,310,000<br>1,200,000<br>[a] 1,250,000 | 0 |
| 71.1° C. (160° F.) | [a] 1,500,000 | 11,700 | 99.20 |

[a] Average.

## SUMMARY AND CONCLUSIONS

(1) The thermal death points of 139 cultures of streptococci isolated from cow feces, from the udder and the mouth of the cow, and from milk and cream showed a wide variation when the heating was performed in milk for 30 minutes under conditions similar to Pasteurization.

At 60° C. (140° F.), the lowest Pasteurizing temperature, 89 cultures, or 64.03 per cent, survived; at 62.8° C. (145° F.), the usual temperature for Pasteurizing, 46, or 33.07 per cent, survived; and at 71.1° C. (160° F.) 2.58 per cent of the cultures survived; all were destroyed at 73.9° C. (165° F.).

(2) The streptococci from the udder were, on the whole, less resistant and those from milk and cream more resistant to heat than those from the mouth of the cow and from cow feces. When heated to 60° C. (140° F.) all of the 18 cultures from milk and cream survived; at 62.9° C. (145° F.) 17, or 94.44 per cent, survived; at 68.3° C. (155° F.) 9 cultures, or 50 per cent, withstood the heating process. All the streptococci from milk and cream were destroyed by heating to 73.9° C. (165° F.) for 30 minutes.

(3) Among the 139 cultures of streptococci there were 22 that formed long chains, which, for the purpose of this paper, were considered as typical streptococci. The others were considered atypical. The typical streptococci were much less resistant to heat then were the atypical.

Of the 22 typical streptococci 12, or 54.54 per cent, survived heating for 30 minutes at 57.2° C. (135° F.); at 60° C. (140° F.) 9, or 40.91 per cent, survived; at 62.8° C. (145° F.) only 1 culture, or 4.54 per cent, withstood the heating. All of the typical streptococci were destroyed by heating for 30 minutes at 65.6° C. (150° F.).

The 117 atypical streptococci were more resistant; at 60° C. (140° F.) 68.37 per cent survived; at 62.8° C. (145° F.) 38.46 per cent survived; and at 71.1° C. (160° F.) 2.56 per cent survived; all were destroyed at at 73.9° C. (165° F.).

(4) Two classes of streptococci seem to survive Pasteurization: (*a*) Streptococci which have a low majority thermal death point but among which a few cells are able to survive the Pasteurizing temperature. This ability of a few bacteria to withstand the Pasteurizing temperature may be due to certain resistant characteristics peculiar to a few cells or may be due to some protective influence in the milk. (*b*) Streptococci which have a high majority thermal death point. When such is the case, the bacteria survive because the majority thermal death point is above the temperature used in Pasteurization. This ability to resist destruction by heating is a permanent characteristic of certain strains of streptococci.

(5) The thermal death point determinations in this work were made in milk in such a manner as to represent actual conditions of Pasteurization by the holder process; therefore the results show what may be expected in commercial Pasteurization, and it is evident that some streptococci may survive the process. However, different results might have been obtained if a larger number of cultures had been studied and if other methods and media had been used for determining the thermal death points.

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# FRUIT-ROT, LEAF-SPOT, AND STEM-BLIGHT OF THE EGGPLANT CAUSED BY PHOMOPSIS VEXANS

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## INTRODUCTION

During the summer of 1912 when searching for eggplants (*Solanum melongena*) affected with stem-rot, supposedly caused by a Fusarium, Mr. G. F. Miles, then pathologist in the Office of Cotton and Truck Disease and Sugar-Plant Investigations, sent the writer some full-grown plants from New Jersey which had the appearance of wilt. The epidermis of the stem for 3 or 4 inches above the soil line was injured and the fibro-vascular bundles blackened. Cultures from the blackened bundles yielded in a few days not a Fusarium but an organism which, because it was isolated from the stem and otherwise agreed with Halsted's description, was regarded as *Phoma solani*.

A disease of the leaf and fruit of the eggplant, commonly attributed to *Phyllosticta hortorum* Speg. has been known to plant pathologists as very prevalent in this country and certain parts of Europe. However, after some study of the organism, Smith (1905, p. 10)[1] concluded that the pycnospores were 2-celled, and proposed the name "*Ascochyta hortorum* (Speg.)." Judging from reports which have appeared since that time, pathologists in general have not accepted the suggested change, but have continued to refer to the organism as *Phyllosticta hortorum* Speg.

The writer regarded the organism on the leaf and fruit as a Phyllosticta and believed that *Phyllosticta hortorum*, *Phoma solani*, and *Ascochyta hortorum* were one and the same fungus. Cross-inoculations were started with *Phoma solani* Hals. and with an organism isolated in 1912 from diseased fruit of eggplants by Mr. A. G. Johnson,[2] of the University of

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 338.

[2] The writer is also indebted to Mr. A. G. Johnson for the use of embedded material and to Dr. I. E. Melhus for the loan of preserved specimens.

Wisconsin, who very kindly gave the writer a culture for comparative studies. The results of inoculation experiments showed that both *Phoma solani* and *Phyllosticta hortorum* are able to produce a fruit-rot and a stem-blight of eggplants. When this fact was determined, morphological studies were made of the two fungi. As a result of this study, the fungi were found identical, and, furthermore, it was concluded that the genus to which they belonged was neither Phoma, Phyllosticta, nor Ascochyta.

## DESCRIPTION OF THE DISEASE

The fungus causing damping-off, or seedling stem-blight, of young eggplants and seedlings was attributed by Halsted (1892, p. 277) to the fungus *Phoma solani.* The stems of seedlings or very young plants attacked by the fungus are girdled for an inch or more above the soil line. The plants soon topple over and die (Pl. XXVI). The part of the stem girdled by the fungus has a smaller diameter than the healthy portion above. This is due partly to the falling away or drying up of the diseased tissue and partly to the arrest in the growth of the stem where the fungus is present. Although pycnidia are usually formed abundantly on the diseased stems of young plants (Pl. XXVII), they develop sparingly or not at all on older ones. They may be developed, however, on old plants by placing the diseased stem for a few days in a moist chamber.

On the leaves (Pl. XXVIII) the fungus causes in the earlier stages brown, dead, round, oval, or oblong spots which become more irregular in shape and jagged in outline with age. The irregularly shaped spots, varying from 2 mm. to 2 or 3 cm. in diameter, are more prevalent on or near the margin of the leaf, or along the midrib or larger veins. These spots consist of a light or grayish inner zone surrounded by a darker, almost black, margin of perhaps ¼ to ½ mm. in width. They are usually not concentrically enlarged, although their appearance sometimes is such. Two or more spots may unite, forming large blotches, and upon the midrib, the petiole, or upon the large veins of the leaf the fungus may produce lesions or abrasions in which pycnidia are formed.

When the fruit is attacked by this organism, it becomes at first soft and mushy, but later mummified and black (Pl. XXIX, fig. 1). A pure culture can usually be secured by planting bits of the inner tissue (Pl. XXX) in plates of agar. Pycnidia may form at first in rather definite spots, but in most cases they will finally cover the whole surface of the fruit. It is believed that young fruit is most subject to attack, although fruit in all stages of growth has been found to be diseased.

The pycnidia, often with a well-defined beak, are at first buried, but later break through the epidermis and appear as brown or black specks extending a little above the surface. On the fruit the pycnidia become visible to the naked eye and are considerably larger than those on the leaves. They stand close together on both the fruit and leaves, separate

in most cases, though occasionally they unite. The pycnidia on the stem are about equal in size to those on the leaf, but they are fewer, except on the stem of very young seedlings. (See Pl. XXVII.) On the leaves the pycnidia vary from 60 to 200$\mu$ in diameter, while on the fruit they measure 120 to 350$\mu$.

Stylospores, the filiform, hooked-shaped bodies, were found abundantly in the pycnidia on the fruit and stems of many plants inoculated in a greenhouse of the Department of Agriculture and on the Potomac Flats, near Washington, D. C. If they were not present on the stem when the plants were lifted, they would frequently develop if kept a few days in a moist chamber. They were also occasionally found in cultures on corn meal.

## INOCULATION EXPERIMENTS

The pathogenicity and relationship of the fungi isolated from the fruit and from the stem of eggplants are shown by the results of inoculation experiments recorded in Table I.

In several experiments the plants were covered for a day or two before spraying and for 24 to 48 hours after with paper-wrapped bell jars or paper-wrapped glass infection cases. This method, however, did not appear to influence the results, since plants which were not covered before or after spraying were likewise infected. In fact, mature plants on the Potomac Flats, near Washington, D. C., were sprayed at 11 a. m. on a very warm, partly cloudy day and left uncovered, and numerous infections of fruit and leaves took place. A few infections were found on the check plants. It is believed, however, that they came from the sprayed plants, since no eggplants were grown within a mile of the experiment, so far as could be determined, and since the check plants nearest those sprayed showed the worst spots. In every experiment with *Lycopersicon esculentum*, *Datura tatula*, and *Capsicum annuum* the plants were covered for 48 hours after spraying.

In all, 27 sets of inoculation experiments have been carried out. Sixty-one eggplants in 7 sets were inoculated by inserting spores and hyphæ of the different organisms into the lower part of the stem, and 59, or nearly 97 per cent, of these plants became infected. Fifty eggplants in 8 sets were sprayed with spores of the different fungi suspended in water, and 47, or 94 per cent, were to some degree infected. Two pots containing many seedlings of eggplants each were sprayed with spores in suspension, and practically all succumbed to the disease. Six half-grown eggplant fruits were sprayed in 2 sets with spores in suspension, and 5 rotted from the effects of the organism. Ten sweet-potato plants (*Ipomoea batatas*) were inoculated at the base of the stem, but none became diseased. Six large tomato plants in 2 sets and 14 small plants in 2 sets were sprayed with a suspension of spores, but none became diseased. Twenty pepper plants (*Capsicum annuum*) in 2 sets and 10 plants of *Datura tatula* in 1 set were sprayed with a suspension of spores, but none were infected.

TABLE I.—*Results of inoculation experiments on eggplant with Phoma solani and Phyllosticta hortorum from various sources*

| Organism No. | Organism. | Name of host. | Age of host. | Place of inoculation. | Method of inoculation. | Number inoculated. | Number infected | Number of checks. | Number of checks infected. |
|---|---|---|---|---|---|---|---|---|---|
| [a] 104 | Phoma solani | Solanum melongena | Half-grown | Potomac Flats | Foliage sprayed with spores suspended in water. | 6 | 6 | 6 | (g) |
| [b] 113 | Phyllosticta hortorum | do | do | do | do | 6 | 6 | 6 | (g) |
| 104 | Phoma solani | do | Mature (fruiting) | Garden near greenhouse. | do | 2 | 2 | 0 | ..... |
| 113 | Phyllosticta hortorum | do | do | do | do | 2 | 2 | 0 | ..... |
| 104 | Phoma solani | do | Medium young | Greenhouse | do | 8 | 5 | 6 | 0 |
| 113 | Phyllosticta hortorum | do | Half-grown | do | do | 10 | 10 | 5 | 0 |
| 104 | Phoma solani | do | Mature | do | do | 10 | 10 | 5 | 0 |
| 113 | Phyllosticta hortorum | do | Seedlings | do | do | Many. | Many. | Many. | 0 |
| 113 | do | Lycopersicon esculentum | Mature (fruiting) | do | do | 3 | 0 | Many. | 0 |
| 104 | Phoma solani | Capsicum annuum | do | do | do | 6 | 0 | 0 | ..... |
| 104 | do | Lycopersicon esculentum | Half-grown | do | do | 8 | 0 | 0 | ..... |
| 104 | do | Datura tatula | Medium young | do | do | 10 | 0 | 0 | ..... |
| [c] 116 | Phyllosticta hortorum | Solanum melongena | Mature | do | do | 6 | 6 | 0 | ..... |
| [d] 109 | Phoma solani | do | Seedlings | do | do | Many. | Many. | Many. | 0 |
| 116 | Phyllosticta hortorum | Lycopersicon esculentum | Mature (fruiting) | do | do | 3 | 0 | Many. | 0 |
| 116 | do | do | Young | do | do | 6 | 0 | Many. | 0 |
| 116 | do | Capsicum annuum | do | do | do | 14 | 0 | Many. | 0 |
| 104 | Phoma solani | Solanum melongena (fruit). | Half-grown | Laboratory | do | 3 | 3 | 0 | ..... |
| 113 | Phyllosticta hortorum | do | do | do | do | 3 | 2 | 0 | ..... |
| 104 | Phoma solani | Solanum melongena | Young | Greenhouse | Spores and hyphæ inserted into the lower part of stem. | 10 | 10 | 6 | 0 |
| 104 | do | do | Mature | do | do | 7 | 6 | 5 | 0 |
| 104 | do | do | Young | do | do | 10 | 10 | 5 | 0 |
| 104 | do | Ipomoea batatas | do | do | do | 10 | 0 | 10 | 0 |
| 113 | Phyllosticta hortorum | Solanum melongena | do | do | do | 10 | 9 | 0 | ..... |
| 109 | Phoma solani | do | do | do | do | 8 | 8 | 6 | 0 |
| [e] 112 | do | do | Half-grown | do | do | 10 | 10 | 5 | 0 |
| [f] 115 | Phyllosticta hortorum | do | Young | do | do | 6 | 6 | 6 | 0 |

[a] Isolated from the dead tissue of a diseased stem of eggplant sent the writer from New Jersey by Geo. F. Miles.
[b] Isolated from the fruit of eggplants by A. G. Johnson, Madison, Wis.
[c] Recovered from the stem of eggplants inoculated in the greenhouse with organism No. 113. Stylospores were present on the plant from which the isolation was made.
[d] Recovered from a plant inoculated with organism No. 104.
[e] Isolated from the stem of a plant inoculated with organism No. 104, on which stylospores were found.
[f] Recovered from the leaves of eggplants sprayed with spores of organism No. 113.
[g] All very slightly.

It is evident from a detailed study of the inoculation experiments that the fungus *Phoma solani* isolated from the stem of eggplant will also infect the fruit and leaves of the eggplant and that the fungus isolated from the fruit (*Phyllosticta hortorum*) will infect the stem and leaves. Both of these organisms will cause a rapid damping-off of eggplant seedlings. Judging from the results of the experiments here performed, both fungi are parasitic on *Solanum melongena* at any age, but not on *Ipomoea batatas*, *Lycopersicon esculentum*, *Capsicum annuum*, and *Datura tatula*. A careful study of infected plants show that the injuries produced by the two organisms are indistinguishable and that the evidence of infection after inoculation is manifested in about the same length of time.

No attempt has been made to recover the organisms from all infected plants. It has, however, been isolated from many infected leaves, fruit, and stems. Many plants have been inoculated with cultures of the organism recovered from previously inoculated plants, and the organism has been recovered a second time from some of these infected plants. In fact, the four cardinal requirements, known as Koch's rules, have been fulfilled in a number of instances as proof of the pathogenicity of the organisms used in the inoculation experiments. In view of results gained from inoculation experiments, it is evident that the two fungi, known as *Phoma solani* and *Phyllosticta hortorum*, are identical. They are also identical morphologically.

## TAXONOMY OF THE FUNGUS

Spegazzini (1881, p. 67) described a fungus occurring on the leaves of *Solanum melongena* as *Phyllosticta hortorum*, the pycnidia of which measured 80 to 90$\mu$ in diameter and the pycnospores 4 to 6$\mu$ long and 2 to 2.5$\mu$ wide. Halsted (1892, p. 279) reported the same fungus on the leaves and fruit of *Solanum melongena* in New Jersey, and at the same time he also reported (1892, p. 277) damping-off or seedling stem-blight of eggplant, which he described as *Phoma solani*. Smith (1904) published a short note to the effect that he had found *Ascochyta lycopersici* on the leaves and fruit of *Solanum melongena*. He says "the fungus differs from *Phyllosticta hortorum* Speg., both in size and septation of spores and in character of leaf spot." He further says that "a careful comparison with Halsted's material showed the two to be distinct"; also that "the spores of *Phyllosticta hortorum* Speg., in material collected by Halsted agree in size with those given by Saccardo, 4–6×2–2.5$\mu$, while those in this Ascochyta are nearly twice that size, 6–12×3.5–4$\mu$." Smith, with the fungus he had, was able to infect *Solanum melongena*, *Lycopersicon esculentum*, *Solanum carolinense*, and *Datura tatula*. The following year he (Smith, 1905, p. 10-14) seems to have thought that the organism he had under observation the year before was *Phyllosticta hortorum* Speg., which "manifested slightly different characteristics from that of the previous year." According to his obser-

vations, "the fungus produced more numerous, as well as more prominent, fruit bodies. The spores were somewhat smaller and the spots lighter colored. In these respects the disease resembled more closely material collected by Halsted and labeled *Phyllosticta hortorum.*" It appears evident from Smith's second article that he regarded *Phyllosticta hortorum* identical with *Ascochyta lycopersici.* If his conclusions are accepted, *Phyllosticta hortorum*, having the priority, would be *Ascochyta hortorum* (Speg.) Smith.

Voglino (1907) in Italy worked with a fungus on eggplants which he thought to be the same as that described by Spegazzini as *Phyllosticta hortorum.* By a series of inoculation experiments with spores of an Ascochyta found on *Solanum melongena* he was able to induce infection on *Physalis alkekengi*, *Solanum nigrum*, *S. dulcamara*, *Lycopersicon esculentum*, *Datura metel*, and *Atrope belladonna.* Voglino agrees with Smith that the fungus previously described by Spegazzini as *Phyllosticta hortorum* is

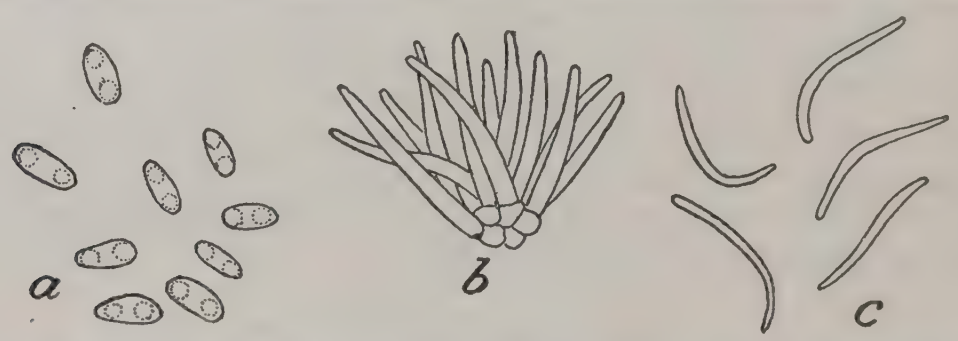



FIG. 1.—Some microscopic characters of the fungus *Phomopsis vexans: a*, Pycnospores; *b*, conidiophores; *c*, stylospores.

an Ascochyta—*Ascochyta hortorum* (Speg.) Smith—and devotes considerable space to a discussion of his reason. To this species must be referred, he says, "*Ascochyta lycopersici* Brun. (*A. socia* Passerini), *A. solanicola* Oudemans, *A. atropae* Bresadola, *A. alkekengi* Massalongo (*A. pedemontana* Ferraris), *A. physalicola* Oud. and perhaps *A. prinzalensis* B. and K."

The writer has made a careful morphological study of the fungus identified as *Phyllosticta hortorum* and collected from the following places: Glen Cove, N. Y. (collected by Pries and identified by Whetzel); Starkville, Miss. (Tracy); Lincoln, Nebr. (Heald); New Brunswick, N. J. (collected by Halsted and identified by Seymour and Earle). The pycnidia in each case were typical of Phomopsis. Specimens collected by Melhus at Madison, Wis., in 1912, already referred to, were examined and found to be a Phomopsis. The pycnidia on specimens from the different localities were more or less beaked, flattened, or irregular in form. They were covered with a thick, black wall (Pl. XXIX, fig. 2) which becomes thinner and less noticeable at the base. The conidia (fig. 1, *a*) were 1-celled, with mostly two, sometimes three oil droplets [1]; the coni-

[1] In rare cases spores might be found, the contents of which appeared divided, but if they were treated with a solution of salicylic acid, the division would frequently disappear, showing that the spores are continuous. The apparent division is merely caused by two vacuoles or oil globules which meet at the center of the conidia.

diophores stout and awl-shaped (fig. 1, *b*). Stylospores (fig. 1, *c*) were found on herbarium specimens from Ithaca, N. Y., on specimens from Wisconsin loaned by Dr. Melhus, and on specimens collected by the writer in New Jersey, on the stem and fruit of inoculated plants, and occasionally in artificial cultures. These characters just mentioned are according to Diedicke (1911) typical of the genus Phomopsis. The spores vary in size from 5 to 8 by 2 to 2.5$\mu$. The morphological characters of the pycnidia on specimens from the various States seem to agree with each other and with specimens produced by the writer as a result of inoculation. A careful study of the fungus with which the writer has worked shows that *Phoma solani* is identical with the fungus causing the fruit-rot and leaf-spot of eggplants and that it belongs to the genus Phomopsis. Attempts to infect *Lycopersicon esculentum*, *Capsicum annuum*, and *Datura tatula* have been unsuccessful.

Owing to the fact that Phomopsis was widely distributed in the United States on *Solanum melongena*, doubt finally arose in the mind of the writer whether *Phyllosticta hortorum* Speg. occurs in this country. Typical specimens of the disease were therefore sent to Spegazzini for examination. After comparing them with his own type specimens, he said that the fungus was not *Phyllosticta hortorum* and pointed out the characteristic differences in the spots, pycnidia, and spores of the two fungi. If, therefore, any value is to be given to a comparison made by an author with his own type specimens, it is safe to conclude that *Phyllosticta hortorum* has not yet been found in this country. How, then, can Smith's results and those of Voglino be explained? The writer is of the opinion that Smith had an Ascochyta on the eggplant, but at the same time another fungus, the so-called *Phyllosticta hortorum*. The writer has examined specimens of Ascochyta on eggplant collected by Whetzel in New York and identified by Jensen as *Ascochyta lycopersici*. The spores are 2-celled and agree in size with spores of *Ascochyta lycopersici*, 6 to 10 by 2.5$\mu$. It is probable that in 1904 Smith had this Ascochyta under observation and in 1905 observed the fungus generally known as *Phyllosticta hortorum*, since he says that the fungus in 1905 produced more numerous as well as more prominent fruit bodies. Such a distinction is certainly true of these two genera as they appear on eggplant. *Ascochyta lycopersici* occurs on both eggplant and tomato, and Smith was able to cross-inoculate these two hosts. The writer, on the other hand, was unable to infect *Lycopersicon esculentum*, *Capsicum annuum*, or *Datura tatula* with the organism he studied. Voglino (1913, p. 213–218) calls attention to a disease of the eggplant, tomato, and pepper which, as a result of cross-inoculations, he believes to be caused by *Ascochyta hortorum*. The spores, however, are considerably larger than those of *Phyllosticta hortorum*, 10 by 3$\mu$, and it is likely that he also had *Ascochyta lycopersici*.

The fungus with which the writer has worked is assigned to the genus Phomopsis because it possesses the following characteristics of that genus:

(1) Stylospores; (2) irregularly shaped or flattened pycnidia, with a well-developed beak; (3) long, stout, and awl-shaped conidiophores; (4) thick, black wall at the top of the pycnidia, becoming less noticeable at the base. It forms a stroma in culture and beaks 1 mm. or more in length.

The combination *Phomopsis vexans* is proposed as the name for the fungus. In 1892 Halsted assigned the name *Phoma solani* to the organism causing the damping-off of the eggplant, apparently not being aware that it was preoccupied by Cooke and Harkness for a fungus on another host. In view of that fact, Saccardo and Sydow substituted *Phoma vexans* for *Phoma solani* Hals.

**Phomopsis vexans** (Sacc. and Syd.), n. comb.

*Phoma solani* Hals., 1892, *in* N. J. Agr. Expt. Sta., 12th Ann. Rpt., 1891, p. 277. nom. nud. Sacc., 1895, Syll. Fung., v. 11, pars 3, p. 490. Not Cooke and Hark., 1884, *in* Grevillea, v. 13, p. 16.

*Phoma vexans* Sacc. and Syd., 1899, *in* Sacc. Syll. Fung., v. 14, p. 889.

*Ascochyta hortorum* (Speg.) C. O. Sm., 1905, *in* Del. Agr. Expt. Sta. Bul. 70, p. 10–14. err. det. Not *Phyllosticta hortorum* Speg.

On the foliage and stems pycnidia loosely gregarious in more or less definite spots, on fruit compact, at first buried, later erumpent, black without, beaked, flattened or irregular in shape, on leaves and stems 60 to 200$\mu$ broad, on fruit 120 to 350$\mu$ broad; pycnospores subcylindrical, somewhat acute, 5 to 8 by 2 to 2.8$\mu$, continuous, hyaline, 2-guttulate, rarely 3; basidia simple, short, straight or slightly curved, hyaline, continuous; stylospores filiform, curved, rarely straight, 13 to 28$\mu$ long.

Hab. on leaves, stem, and fruit of *Solanum melongena*. Type specimens deposited in the herbarium of the pathological collections of the Bureau of Plant Industry, Department of Agriculture, Washington, D. C.

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## PLATE XXVI

Damping-off of seedling eggplants which had been sprayed with organism No. 113 (*Phyllosticta hortorum*). All the plants were finally killed by the fungus.

## PLATE XXVII

Seedling eggplants from the pot shown in Plate XXVI (enlarged), showing the presence of pycnidia and the effect of the fungus on the stem.

## PLATE XXVIII

An eggplant leaf sprayed with organism No. 104 (*Phoma solani*), showing the characteristic spots and pycnidia formed therein.

## PLATE XXIX

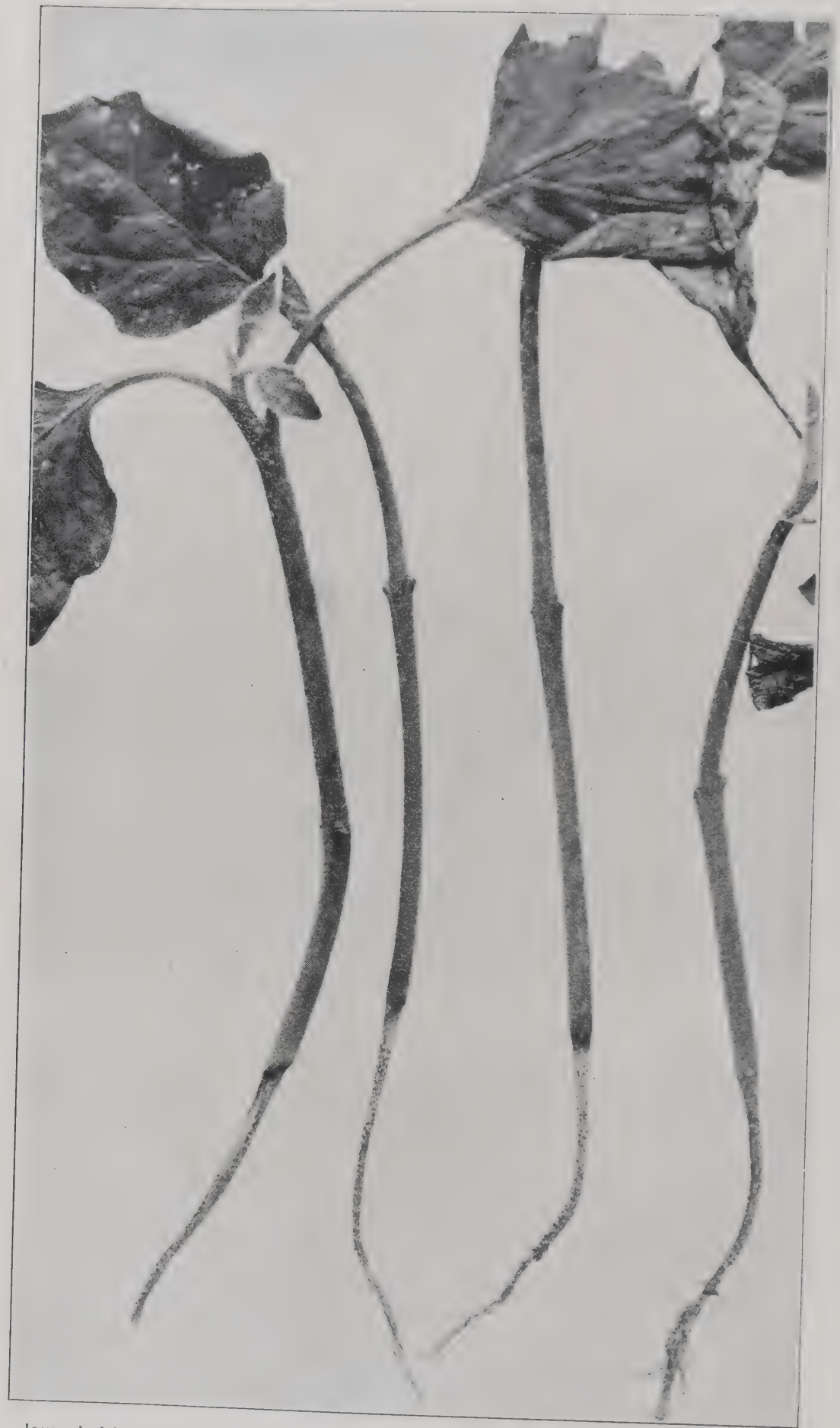
Fig. 1.—An eggplant fruit produced by a plant grown on the Potomac Flats and mummified as a result of spraying with organism No. 104 (*Phoma solani*). Note the pycnidia on the surface.

Fig. 2.—A photomicrograph of a cross section through pycnidia formed on the calyx of an eggplant. Note the thick black wall covering the pycnidia.

## PLATE XXX

A section through the fruit of an eggplant which had been sprayed in a moist chamber with organism No. 113 (*Phyllosticta hortorum*). The fungus had entered and softened the fruit to the depth shown by the darkened portion of the photograph and was recovered in pure culture from the rotted interior. Other fruits similarly treated and left longer in the moist chamber were completely rotted.

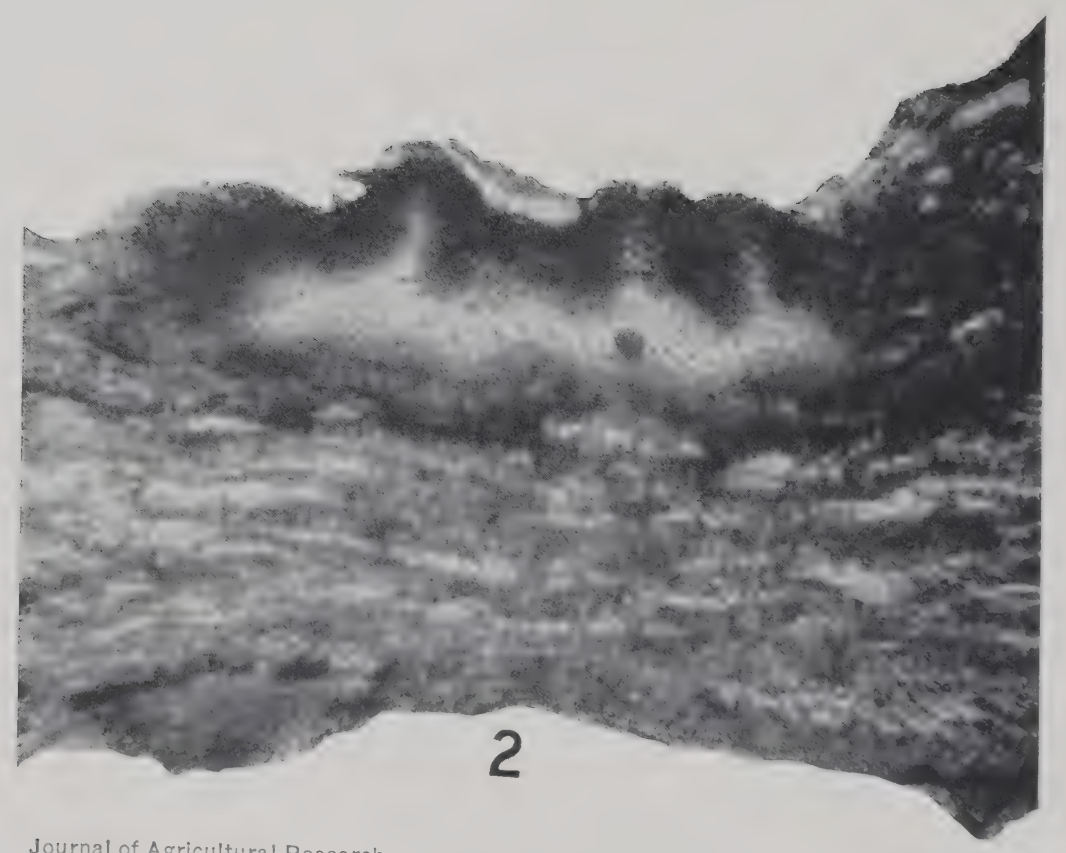




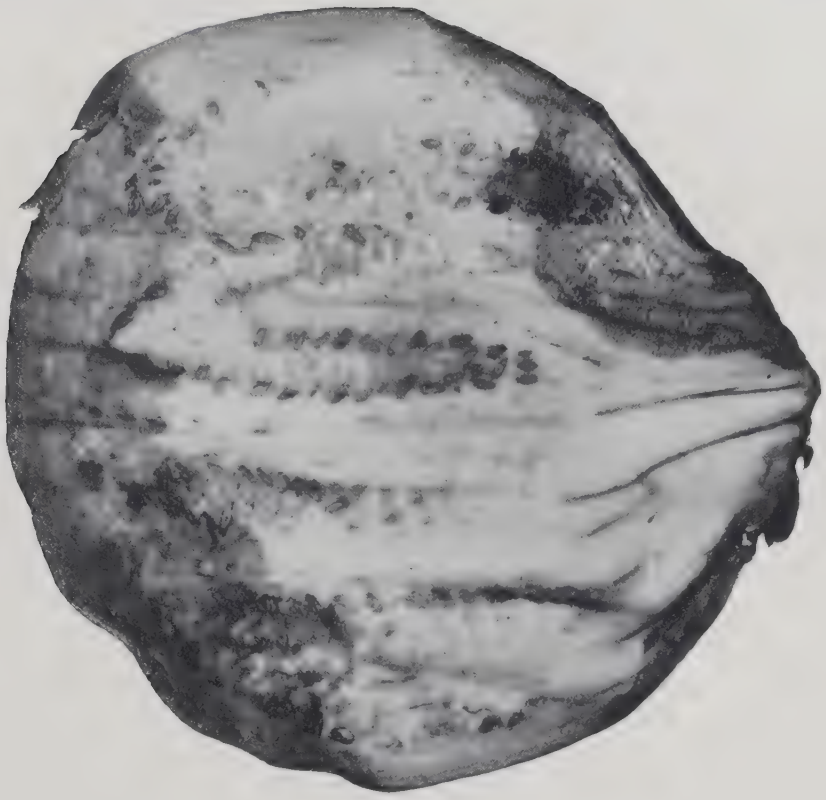


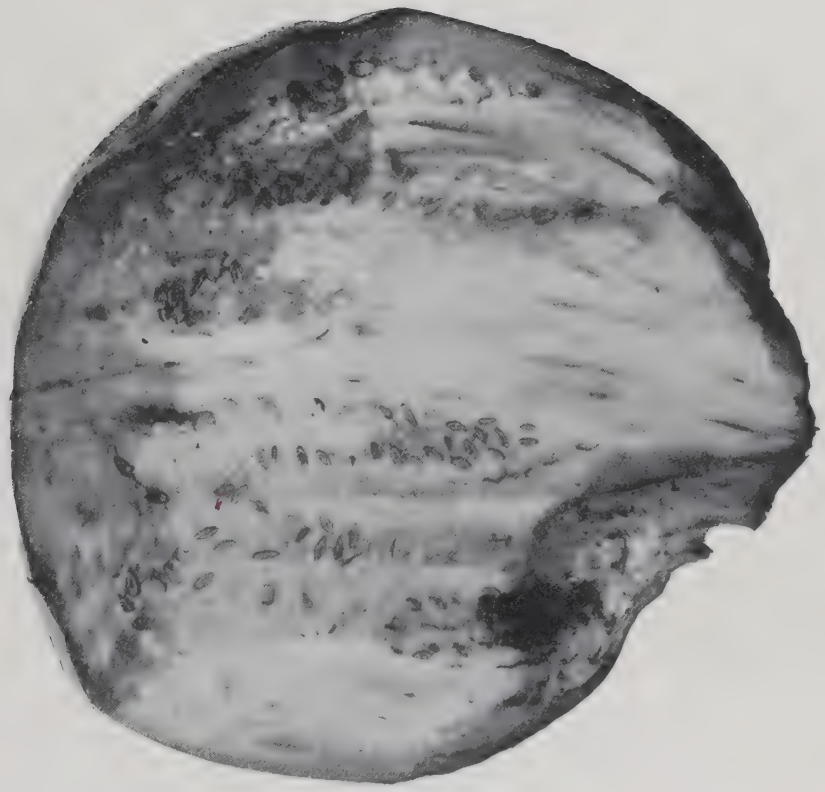


1



2





# HEAD SMUT OF SORGHUM AND MAIZE

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## GENERAL CHARACTERISTICS OF THE DISEASE

### DISTRIBUTION

In the agriculture of western Kansas and Texas and similar parts of the Great Plains area various sorghum varieties have recently attained considerable importance as a dry-land crop in the farming operations which are developing in the sections formerly devoted to cattle ranges. This fact, together with the importance of broom corn in some sections, has led to an investigation of the diseases of the sorghum crop by the Office of Cereal Investigations of the Bureau of Plant Industry.

The study of the head smut has an added importance from the fact that it occurs on maize (Indian corn) and has been reported by McAlpine (1910, p. 290)[1] as serious on that crop in Australia, and by Evans (1911) and Mundy (1910, p. 1) in South Africa (Pl. XXXI). It has been found on maize in some abundance in this country (Norton, 1895; Hitchcock and Norton, 1896, p. 198), although the writer, in rather extensive observations, has never seen such a case; nor has it been recently reported.

The parasite is widely distributed in sorghum-growing regions throughout the world, and in some sections, chiefly tropical or subtropical, it is very destructive. Munerati (1910, p. 718) has found it abundant on *Sorghum halepensis*, and it has also been reported from Italy by Passerini (1877, p. 236), Mottareale (1903, p. 3), and Cugini (1891, p. 83); from India by Cooke (1876, p. 115) and Barber (1904); from Egypt by Kühn (1878, p. 10); from German East Africa by Busse (1904, p. 378); and from Japan by Hori (1907, p. 163). According to Hennings (1896, p. 119), it occurs in North and East Africa, Madagascar, and East India, as well as in Central and South Europe. While it has been reported from Iowa, Illinois, Kansas, Minnesota, Mississippi, Nebraska, New Jersey, Ohio, and Texas, according to Clinton (1904, p. 393), it is fortunately still quite rare in this country. Clinton states that it was probably introduced into the United States with importations of sorghum seed from Europe. This seems quite possible in considering Kellerman and Swingle's (1890, p. 159) original note on its occurrence in this country, where it is noted that it first occurred in New Jersey on Amber sorgo (sweet sorghum). In Kansas it was first noticed on "Red Liberian" (sumac)

[1] Citations to literature in parentheses refer to "Literature cited," p. 369–371.

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sorgo (Failyer and Willard, 1890, p. 145), which would suggest Africa as its source.

There appear to be three distinct forms of smut (Pl. XXXII, fig. 1) affecting the sorghum crop in America (Potter, 1912): *Sphacelotheca cruenta* (Kühn), *Sphacelotheca sorghi* (Link) Clint., and *Sorosporium reilianum* (Kühn) McAlp., the head smut (Pl. XXXII, fig 2). Of these the last-named alone has consistently resisted efforts to prevent its spread, though all known methods for the prevention of cereal smuts have been tried. The serious occurrence of the disease has been observed to be confined at present to the Texas Panhandle. For this reason the investigations, begun in 1907 by Dr. E. M. Freeman and continued after 1909 by the writer,[1] have been carried out chiefly at Amarillo, Tex., with plantings at other points for comparison. This work has been supplemented by studies in the greenhouse and laboratory at Washington, D. C.

## SYNONYMY

The head smut of sorghum was first noted by Julius Kühn (1875), who described it from a specimen sent to him from Egypt by Dr. Reil in 1868.[2] The mistake he made in describing the spores as smooth was repeated by Passerini (1876) when he described the form of maize. The echinulations are often obscure, however, unless the spores are quite mature and dry. Brefeld (1883, p. 94) describes them as almost smooth.

Saccardo (1876) and de Toni (1888) described this smut as showing an aggregation of spores suggestive of Sorosporium, as did also Norton (1896, p. 233). Busse (1904, p. 381) suggests in this connection, as Brefeld (1883, p. 171) did earlier, that possibly the genus Sorosporium should not be retained. Busse notes and figures the characteristic spore aggregates, but states that this smut is intermediate in this respect between Ustilago and Sorosporium. According to Dietel (1900, p. 7), the two genera are not sharply distinguishable. Although the spores are rather loosely bound together in this species, McAlpine (1910a, p. 181) has recently placed it in the genus Sorosporium. Under the present artificial system necessitated by a lack of adequate knowledge of the natural relationships

[1] The author wishes to acknowledge the advice and assistance of Mr. E. C. Johnson, who was in charge of the cereal-disease work from 1908 to 1912, inclusive, during which time most of the work here presented was done. Considerable assistance has also been given by various officials at the stations where the work was performed, among whom Dr. E. M. Freeman should be especially mentioned.

[2] "*Ustilago Reiliana* Kühn in litt. U. sporis laevibus, subglobosis, crassiusculis (10, 4 Mikr. inter et 13, 3 Mikr. diamet. variantib.) semipellucidis, brunneis; paniculam totam contractam et obvolutam et abortivam corrumpens. Crescit in Sorgho vulgari." Rabenhorst's Fungi Europaei Exsiccati, No. 1998.

The name given by Kühn is still retained by European mycologists. Its synonymy follows:

*Ustilago reiliana* Kühn, 1875, *in* Rabenh., Fungi Europ. Exs., ed. nova, s. 2, cent. 20, no. 1998.

*Ustilago reiliana*, forma *zeae*, Pass., 1876, *in* Rabenh., Fungi Europ. Exs., ed. nova, s. 2, cent. 1 (resp. cent. 21), no. 2096.

*Ustilago pulveracea* Cooke, 1876, *in* Grevillea, v. 4, no. 31, p. 115, pl. 63.

*Cintractia reiliana* Clint., 1900, Ill. Agr. Exp. Sta. Bul. 57, p. 346.

*Ustilago (Cintractia) reiliana* forma *foliicola* Kellerm., 1900, *in* Ohio Nat., v. 1, no. 1, p. 9, pl. 2.

*Sphacelotheca reiliana* Clint., 1902, *in* Jour. Mycol., v. 8, no. 63, p. 141.

*Sorosporium reilianum* McAlp., 1910, Smuts of Austral., p. 181.

in this group this classification seems proper in view of his illustration (pl. 30, fig. 37) and of our Plate XXXIII. From these it is evident that the spores, as they occur aggregated into irregular groups, are so formed in the sorus, for the spore balls are found before the spores are mature or even before the latter are differentiated—i. e., while the fungus is still in the hyphal stage.[1]

## GROWTH IN ARTIFICIAL CULTURES

The recent work of Appel and Riehm (1911, p. 346, pl. 4[2]) has again emphasized the fact, first established by Brefeld, that the smuts can be cultivated on artificial media in their saprophytic stages. Similar work with this organism has been found difficult on account of trouble in collecting spore material free from contamination and thoroughly germinable. Indeed, the writer has rarely succeeded in getting over 15 per cent of the spores to germinate. The large, open sorus, moist with the saccharin juices of the host, gathers yeasts, molds, and bacteria, which are very troublesome, particularly in liquid cultures. These were attempted repeatedly in several different seasons and at various times of the year, but with only slight and irregular germinations, no matter what the age, source, or condition of the spores. Cane-sugar solutions were largely used, as well as distilled water, rain water, tap water, soil decoctions, sorghum sap, beef bouillon, decoctions of carrots and of prunes, Uschinsky's solution, and Cohn's solution, the last named being also tried in the modified form used by Hitchcock and Norton (1896, p. 200) in their work with this smut. The temperatures were not controlled or recorded in most cases.

With solid media, however, the isolation of the spores found germinating was accomplished by transplanting them with glass hairs under the binocular microscope to sterile poured plates, where their development into conidial colonies was watched under the microscope. Plates seeded thinly enough to contain few contaminations would so seldom show any germinating spores that transplanting from a thickly seeded plate proved to be the only practicable method of isolating, since the head-smut colonies developed so slowly at ordinary temperatures (over a week was required after germination for the colony to become visible to the naked eye) that the plates would be obscured by other organisms long before the smut could be isolated in the usual way. Moreover, the method employed made it certain that the conidia thus obtained in pure culture were not those of some contaminating yeast. It should be said, however, that since this was done it has been found that the yeast and bac-

[1] The character of the sorus, particularly in the decided deformity of the whole inflorescence, also seems more closely similar to several of the species of Sorosporium than to any of Sphacelotheca as described by Clinton (1904, p. 383–395). Although the observations here presented do not appear to be in accord with the classification given this form in Clinton's monograph, the writer is much indebted to Dr. Clinton for helpful criticism.

[2] Erroneously marked "plate 3."

terial contaminations (not the molds) can be almost entirely eliminated without injury to the spores by treating with copper sulphate (see p. 356–357).

The isolation of the organism gave excellent opportunity for a closer study of its relation to various media and temperatures. Plate XXXIV, fig. 1, shows its growth in about six weeks from transfer on carrot agar at 20° to 23°, 30°, 35°, and 40° C., respectively. At 40° there is no growth. At 35° the growth is very slight, light brown in color, and much attenuated. A culture at 32.5° C. grew poorly, and those at higher temperatures were eventually killed, for they did not grow on being removed from the incubator. The rapid development at 30° indicates that this is very near the optimum temperature for the organism, and this is borne out by the studies of germination given in Table I.

TABLE I.—*Germination of spores of the head-smut organism at various temperatures*

| Serial No. | Date of test. | Temperature. | Duration of test. | Germination. |
|---|---|---|---|---|
| | 1912. | °C. | *Days.* | *Per cent.* |
| 1 | Dec. 16 | 29–31 | 3 | 6.0 |
| 2 | ...do..... | [a]20–21 | 3 | 2.0 |
| 3 | ...do..... | 16–20 | 2 | .2 |
| 4 | ...do..... | 17 | 3 | .2± |
| 5 | ...do..... | 14.5 | 3 | 0 |
| 6 | ...do..... | 12 | 3 | 0 |
| 7 | ...do..... | 8.5 | 3 | 0 |
| 8 | ...do..... | 7.5 | 3 | 0 |
| 9 | ...do..... | 4 | 3 | 0 |
| 10 | ...do..... | 1 | 3 | 0 |
| | 1913. | | | |
| 11 | Jan. 8 | 40 | 3 | 0 |
| 12 | ...do..... | 37.5 | 3 | 0 |
| 13 | ...do..... | 35 | 3 | 0 |
| 14 | ...do..... | 32.5 | 3 | 1.5 |
| 15 | ...do..... | 30 | 3 | 7.9 |
| 16 | ...do..... | [a]23–25 | 3 | 3.0 |
| 17 | ...do..... | [a]20–23 | 3 | 1.0 |
| 18 | ...do..... | 18–20 | 3 | 2.0 |
| 19 | ...do..... | 20–23 | 3 | 5.0 |
| 20 | Mar. 18 | 27 | 8 | 4.0 |
| 21 | ...do..... | 17 | 8 | .4 |
| 22 | ...do..... | 9 | 8 | 0 |
| 23 | ...do..... | 23 | 8 | 2.0+ |
| 24 | Mar. 19 | 27 | 7 | 13.1 |
| 25 | ...do..... | 17 | 7 | 1.0 |

[a] All but these were incubated in the dark.

These germinations were made in carrot-agar plates with material collected at Amarillo, Tex., in September, 1911, from Red Amber sorgo, except the last two, which were from kafir grown in 1912. From Nos. 11 to 19, inclusive, the number of spores counted in each case was 200; for the rest of the tests the count was not recorded except as follows: No. 20, 1,000; No. 23, 500; No. 24, 541; and No. 25, 818.

In respect to its optimum temperature, then, the head smut is quite unlike those smuts which infect chiefly from seed-borne spores.[1] It is, on the other hand, closely similar to those infecting intraseminally—i. e., the loose smuts of barley and wheat (Appel and Riehm, 1911, p. 364)—and also seems to resemble corn smut, *Ustilago zeae* (Beckm.) Ung., which, while infecting extraseminally, has a late period of infection and shows a more or less localized development. Preliminary observations on corn smut indicating a similar relatively high optimum temperature were made at the same time as Nos. 11 to 19, inclusive, in Table I; and it is this analogy, rather than that with the loose smuts, which has been supported by the evidence of inoculations and other experiments, presented later.

The fact that the head smut is indigenous to a host from subtropical climates should also be pointed out in this connection. At low temperatures, however, the organism can not be said to be injured, although it grows very slowly, if at all. Even severe freezing does not kill it. Both the spores and conidia have been frozen at St. Paul, Minn., at outdoor temperatures which reached a minimum of −26° C., in both a wet and dry condition, and some were still found to be viable, though frozen for over three weeks. Similar tests at Amarillo, Tex., and at Washington, D. C., were generally confirmatory of these results, although much weathering sometimes appeared to destroy viability.

The writer has not found the spores readily germinable after several years, as did Brefeld (1883, p. 95). Furthermore, the conidia have not survived periods of drying, lasting from four to eight months at ordinary summer temperatures. The method used for determining the latter was to smear some cover glasses with conidia from carrot-agar culture and leave in a Petri dish or culture tube for the period mentioned before transferring to a culture medium for test of viability.

The organism has been found to develop well on malt extract and beerwort agars—perhaps even better than on carrot agar. A synthetic dextrose agar is also favorable. Plate XXXIV, figs. 2 and 3, shows the characteristic, rugose conidial growth. Carrot agar gives a more rapid growth, but the darkened central area of the culture shown in Plate XXXIV, fig. 3, is becoming brown. This may be caused by differences in drying or by the influence of contaminations near it in the plate. A malt extract prepared from germinated Amber sorgo seed was tried, but did not prove to be as favorable a medium as the others. On a 3 per cent cane-sugar agar the growth was scant. Gelatin is liquefied readily. While the organism grows well in 1 per cent peptonized (1 per cent of peptone) solutions of saccharose, lactose, levulose, dextrose, and maltose,

[1] See Herzberg (1895, p. 23) on *Ustilago avenae*. Dr. H. B. Humphrey, at present pathologist in the Office of Cereal Investigations, has found in unpublished experiments that *Tilletia tritici* has an optimum temperature of very close to 20° C.

it does not ferment any of them. Spores, or decidedly sporelike bodies [1] (Pl. XXXIV, fig. 4), are frequently formed in liquid cultures, which then show the brown color characteristic of the resting stage. These may also be found occasionally in agar cultures. They are usually undersized (7.5 to 12$\mu$) and show only traces of echinulations. Their germination has not been observed. In the upper part of the figure (Pl. XXXIV, fig. 4) are shown some of these artifically grown chlamydospores (on the left) with natural spores (on the right) for comparison. Below are shown chains of spores and examples of peculiar formations which are suggestive of the involution forms in many bacteria.

## FLORAL ALTERATIONS

A peculiar reaction between this parasite and the host manifests itself by a vegetative stimulus to the host, not only in the vegetative parts but also in the inflorescence.

The parasite of head smut does not always develop a sorus on an infected culm, but frequently causes a floral sterility (Pl. XXXV, fig. 1) which develops at times into a peculiar proliferation of the panicle (Pl. XXXV, fig. 2). This phenomenon, in the tassels of maize, has already been noted and figured by Hitchcock and Norton (1896, p. 199). In extreme cases of this sort in sorghum (Pl. XXXV, fig. 2) the ovary and stamens entirely disappear and the growth takes the form of a complete individuation in the place of each flower; a tiny culm, with leaves, nodes, and rudimentary panicle, shoots up from the head almost as if in an effort to escape the parasite. The hyphæ of the latter were found in one instance to have penetrated the tissues of the phyllomorphic or almost phytomorphic flower (Pl. XXXVI). They are distinctly shown in the illustration as darkly stained threads in the upper part of the panicle and in the bud at its base. In some of the parenchymatous tissue the nuclei are abnormal and have taken the stain like the hyphæ. A number of other flowers less strongly proliferated were examined and found to contain no hyphæ. It may be concluded from this that the change is probably caused by alterations in nutrition processes, especially since a somewhat similar though less pronounced phyllomorphism has been observed in districts where the head smut does not occur, as at Arlington, Va. (Kusano, 1911).

Where the smut occurs commonly, however, this proliferation of the inflorescence is very characteristic and furnishes a more ready means of distinguishing the infected plants than the presence of the sori themselves. Indeed, of 125 plants of Red Amber sorgo examined in three different seasons (1910, 1911, and 1912), mostly at Amarillo, only two

[1] Brefeld (1883, p. 158) obtained the spores of *Tilletia tritici* in artificial culture and Busse (1904, p. 375) has done so with another sorghum smut, *Ustilago cruenta* Kühn. He did not culture the head smut, doubtless because of the interference of contaminations which he mentions (p. 377). Grüss (1902, p. 219) has described spore formation in *U. zeae* in cultures. Herzberg (1895, p. 7) does not consider them analogous to those formed on the host, although he germinated some of them in the case of *U. tritici*.

were found to be wholly smutted—i. e., producing spores in every head. Infected plants of this variety almost always have some normal culms, although the number of these varies greatly with the season. Of the 125 plants examined, 64, or more than 50 per cent, produced one or more culms with normal panicles. An infected culm may bear a normal head, but this is rare. Usually such a culm bears no seed, and there is almost always some degree of abnormality in evidence, the glumes becoming elongated and either decolorized or of a greenish hue.

### INFECTION OF NODAL BRANCHES

Along with these floral changes there usually occurs an abnormal tendency to branch. Indeed, the development of the buds, which occur alternately on opposite sides of the culm at each node, much as in other Gramineae (Hackel, 1887, p. 3), is often the only positive evidence of the infection, since the resulting branches usually bear sori. This phenomenon has led Busse (1904, p. 386–392)[1] to consider the infection of a branch to take place from hyphæ within the node, growing up through the tissue of the sheath at the time the bud begins to develop, and he evidently concludes (p. 391) that these nodal buds are not infected until they begin to grow out into branches. The histological data given in support of his view seem inadequate to establish, beyond a question, his identification of smut hyphæ in the lesions which sometimes occur in the sheath over the swollen buds. The present investigation has shown, too, that these buds become infected without reference to their development into branches and that there is a peculiar regularity about the infection even when some of the branches are missed.

Forty culms from 15 infected plants of Red Amber sorgo (S. P. I. No. 17548) grown at Amarillo were dissected and studied for the occurrence of the parasite in the nodal buds, and the results are summarized in figure 1. The material was killed and fixed with aceto-alcohol (Carnoy's fluid), a mixture of one-third of glacial acetic acid and two-thirds of commercial alcohol, for periods varying from 2 to 24 hours. It was then rinsed in two or three changes of 70 per cent alcohol and kept in this until embedded in paraffin in the laboratory at Washington, D. C. All the buds from a single culm were prepared and kept together in one vial and were distinguished from each other by cutting them into different shapes, which were sketched into a record showing their position on the culm.

The oft-recurring difficulty in definitely differentiating between the host and parasite by staining methods was encountered in this work. After experimentation it was found that this organism is Gram-positive under most conditions, and with a counterstain of eosin in clove oil a very

[1] Busse (1904, p. 391) says, "Ich nehme an, dass die Infektion nicht direkt, sondern auf dem Umwege über die mit dem Stengel organische verbundene Hauptsprossscheide zu stande kommt." *See also* his Pl. V, figs. 15, 18, 18c, and 19.

sharp contrast was obtained. This proved to be a quick, convenient method, and the stain is fairly permanent if the clove oil is carefully washed off with xylol before mounting in balsam.

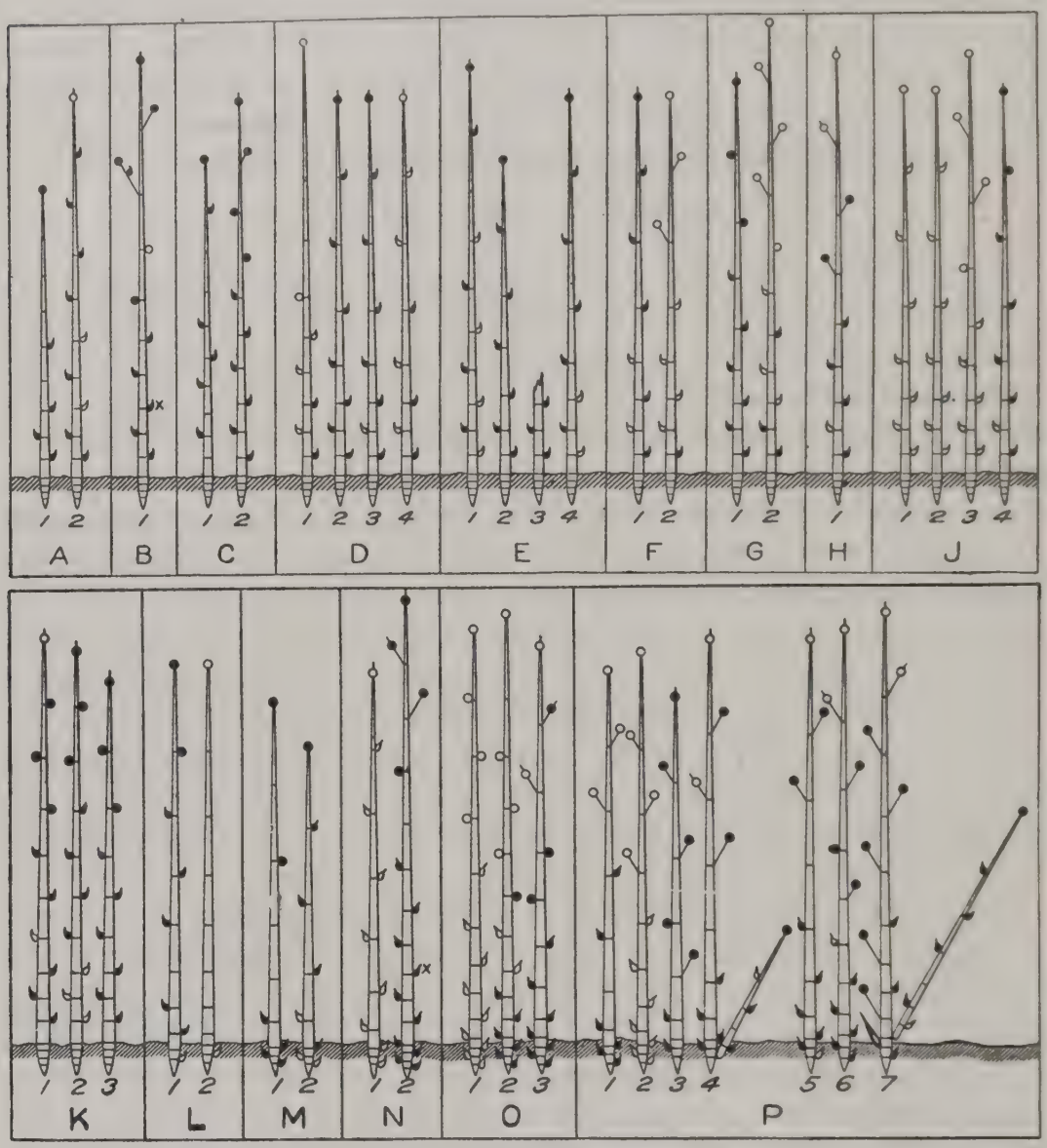



FIG. 1.—Diagrammatic representation of the occurrence of infection in the nodal buds or branches of several sorghum plants.

In figure 1 each plant is designated by a letter and its culms by numerals. The culms are represented with nodes and with branches where they occurred, but without leaves, sheaths, or roots. The growth at each node is represented as follows: A bud which has developed a panicle, either directly evident, as in the main inflorescence, or rudimentary and discovered in dissecting, is represented by a circle, while buds developed to a lesser degree are represented by a subovate symbol. Those showing spore development or, upon microscopic examination, the presence of the hyphæ of the parasite, are shown in solid black, while those which were normal are in outline. In cases where the panicle was not completely parasitized or where the inflorescence, while showing no spore formation, was wholly or partially sterile, the culm is represented as extending through it, the presence or absence of spore formation being indicated as above. When no growth is represented at a node, it signifies that the bud was lost in handling or that for some other reason it was not examined.

All the plants represented in figure 1, except O, were dissected in the early autumn of 1910 at Amarillo. Plant O was one of a number prepared in 1911. In plants A to K, inclusive, no buds were taken from below the surface of the ground. In all cases, however, the exact position of the ground line was not recorded, but has been assumed. The buds on the suckers shown in plant P were not necessarily situated as shown, since they were too small to differentiate by the method used. Culm P3 also bore a sucker at the first node, on which three buds were infected and three apparently undiseased, the apical bud being lost.

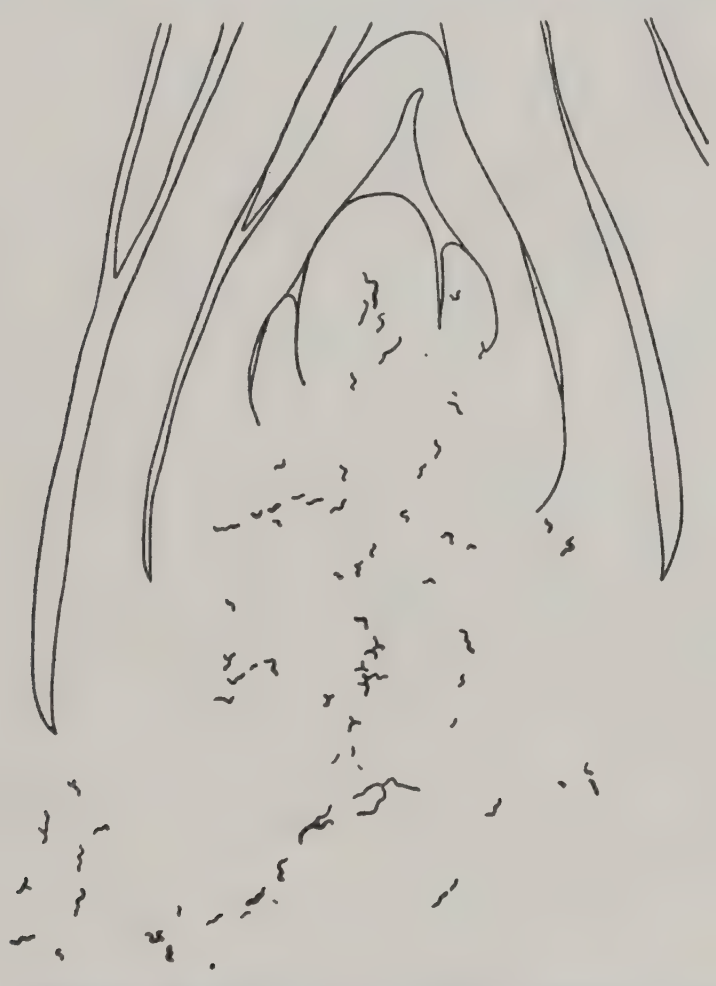


FIG. 2.—Diagram of Plate XXXVII, figure 1, showing the position of the hyphæ.

An examination of the diagrams reveals the fact that most of the culms were but partially infected. A particularly noticeable feature is that when only a few of the buds were missed by the parasite they occurred neither at consecutive nodes nor yet irregularly, but almost without exception included only such as were on the same side of the culm. This is well illustrated in culms A2, D3, E1, E4, F1, J4, and L1. In the same way, if only a few of the buds were involved in the infection, they, too, were usually on the same side of the culm and at the base of the plant, as seen in culms D1, D4, and F2. The basal portion sometimes escaped (as in culms K2 and M2), and occasionally the top grew away from the

parasite (as in culms O2, O3, P1, P5, P6, and P7), though usually remaining sterile. Thus, the plant is seen to have been infected only in such of the buds as were developed from a definite section of the original meristem. The few irregularities (culms G2, H1, K1, O2, and P6) can not be said necessarily to conflict with this interpretation, but were probably the result of unusual developments, such as a double infection, or, perhaps, of errors in technique or records in repeatedly handling these 300 or more buds. It seems certain that the dominance of cases showing regularity of infection can not be due to error.

Plate XXXVII illustrates the appearance of the hyphæ in two of these nodal buds. The two buds in question are marked by a cross in text figure 1. In Plate XXXVII, figure 1, the host tissue was stained more deeply than in the other, and the hyphæ, which are intercellular, do not show as well, particularly those not exactly in focus. Text figure 2 will assist in locating such as are discernible in Plate XXXVII. It should be noticed that in this section the hyphæ are seen mostly in the tissues on the left, while in the other nearly all of them are on the right. Such an arrangement doubtless occurred in the buds from which such infections developed as are shown in culms A2, D4, E1, F1, etc.

It is apparent that no assumption of the occurrence of the primary infection at or near the maturity of the host can explain the regularities of the infection phenomena usually found in these buds without also assuming an improbable spread of the infection in the mature tissues of the host. The nodal branches were evidently infected early, when the buds formed, if at all. As Brefeld (1895, p. 47, 84) observed in connection with his work on infection with *Ustilago cruenta*, the sorghum plant grows very slowly at first for a period of about four weeks or more. It was during this time, then, while the meristem, at least in each culm, was confined to a comparatively small compass, that the spread of the infection must have proceeded in such a way as to determine its later development in these plants.

## LIFE HISTORY OF THE PARASITE

### PREVIOUS WORK

That the head smut infects its host in the early seedling stage has been the general assumption as to its life history, although the results of inoculations performed by investigators would seem to have given doubtful support to the idea. Brefeld (1883, p. 94) states that Kühn, who named this parasite, obtained a double, artificial infection with this smut and *Ustilago cruenta*. Passerini (1877, p. 236) says he was able to reproduce the head smut on maize, but not on sorghum. W. A. Kellerman (1891, p. 98, 101) produced slight infection in greenhouse and field experiments by inoculating the seed. Later (1900a, p. 9)[1] he

[1] See "Literature cited" for notes published in 1898; with K. F. Kellerman in 1899; and with O. E. Jennings, reporting further negative results, in 1902.

produced it also on maize and described the form *foliicola*. While he states (1900, p. 18) that infection from seed-borne spores takes place and that, therefore, seed treatment with fungicides is of value, he had, like Passerini, produced the disease, in the field, only on maize and in very small quantities. Clinton (1900, p. 347) also failed to produce any infection by inoculations of the seed and young plants. Hori (1907, p. 163, 166) reports entirely negative results from inoculations, but claims that a hot-water treatment has been shown to prevent the disease. McAlpine (1910, p. 296) produced infection in a single maize plant by seed inoculation and on this basis recommended seed treatment with copper-sulphate solution as a preventive. Johnston (1910 or 1910a, p. 44) has also recommended seed treatments, and this Australian idea has been copied by Mundy (1910, p. 4) in South Africa.

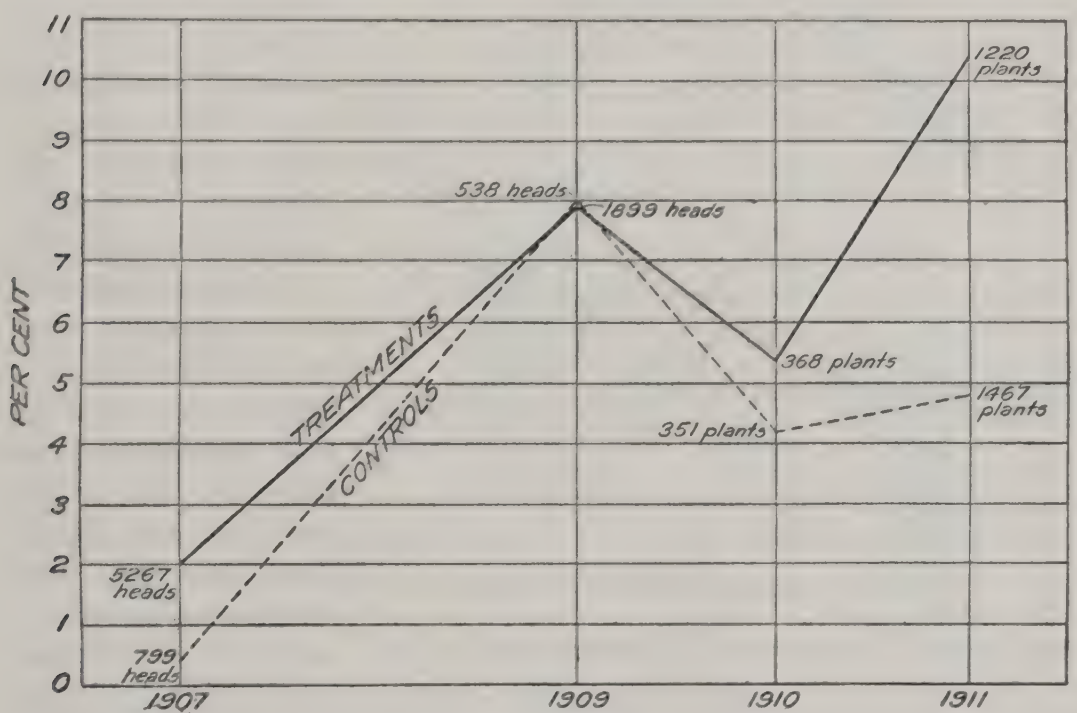



FIG. 3.—Curves summarizing for different years the percentages of infection in plantings of sorgo after all hot-water treatments and in control plantings.

The early inoculation experiments of the Office of Cereal Investigations, involving about a thousand plants of different varieties (including kafir and sorgo) in the field at Amarillo, gave results similar to those cited above—i. e., little or no infection resulted from the presence of an abundance of spores on the seed.

## SEED AND SPORE TREATMENTS

In full accord with the negative results of these inoculations our experiments have conclusively shown that the most severe treatments of the seed, though carefully performed, do not prevent the attack of the parasite. These treatments have involved some 35,000 or more plants, of which about two-thirds were in tests of thermal methods, the

rest of the tests being performed with fungicides. For the latter, formalin, copper sulphate, cresol, and potassium sulphid were tried. Kafir, broom corn, and sorgo were used, and of these the first two developed so little infection that the results were of no significance.

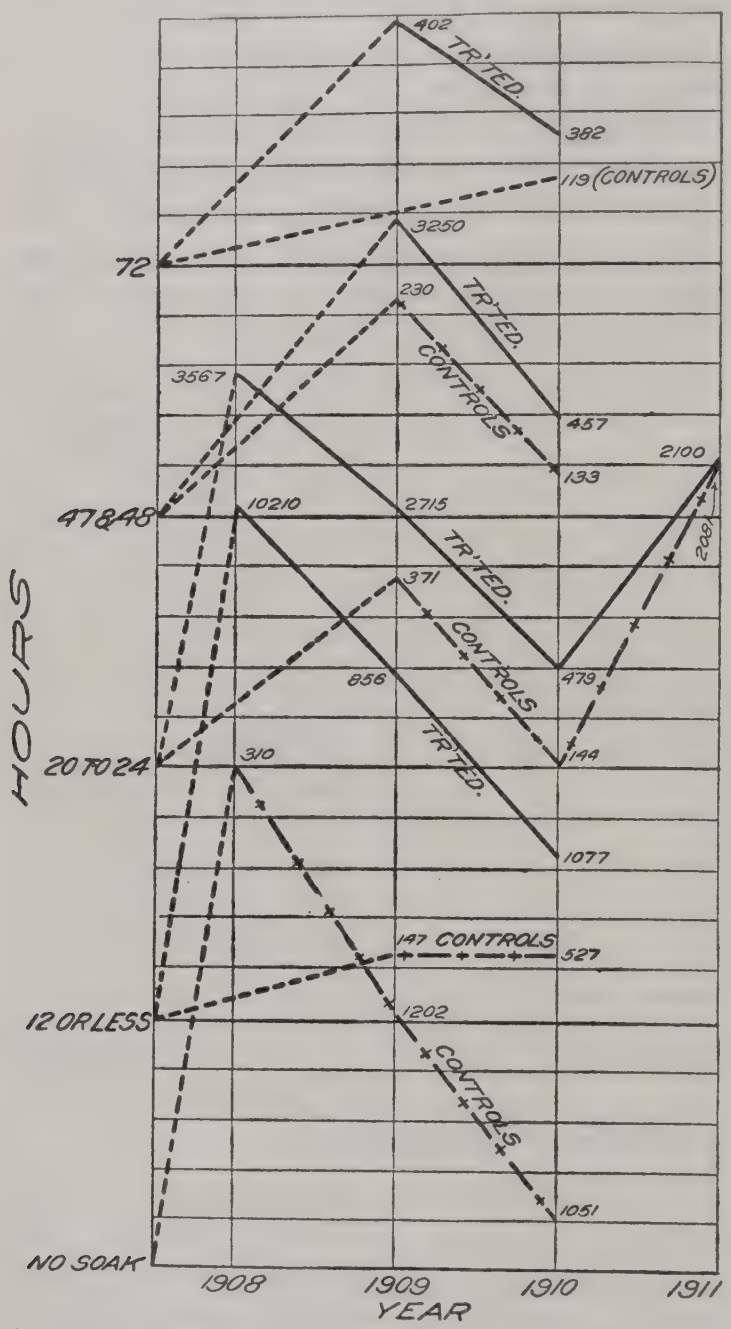



FIG. 4.—Curves summarizing for different years the percentages of infection: First, in plantings of Red Amber sorgo after modified hot-water treatments at all temperatures and of all durations, but after presoakings of various duration; and, second, in control plantings (not treated with hot water).

With the more susceptible sorgos (chiefly the Red Amber variety), however, quite heavy infections occurred in some seasons. The important features of the results are brought out in the summaries presented in figures 3 to 7, inclusive. The first and last of these figures present results obtained with several varieties of sorgo, the one being a summary of treatments performed with hot water without presoaking and the other a summary of the whole work on seed treatments, including both thermal and chemical methods. The three others (figs. 4, 5, and 6) show the results of modified hot-water treatments [1] of Red Amber sorgo (S. P. I. No. 17548) according to the three elements of the treatment: figure 4, according to the length of presoaking given the seed; figure 5, according to the duration of the hot-water treatment; and figure 6, according to its temperature.

[1] This method was originated by Jensen (1888). See Freeman and Johnson (1909) and Appel and Riehm (1911). Tepid water for presoaking was tried in a few of these treatments of sorghum, but without any difference in results.

In summarizing the results for constructing these curves, the duration of presoaking in the modified treatments and the duration and temperature of treatments have been approximated in several instances in order to bring all of them to intervals of 12 hours of presoaking, 5 minutes in duration of treatment, or 2 degrees in temperature. The results of treatments performed in 1909 and previously were recorded by counting heads, while subsequently they were recorded by noting the number of plants. These numbers are given at each point in the curves.

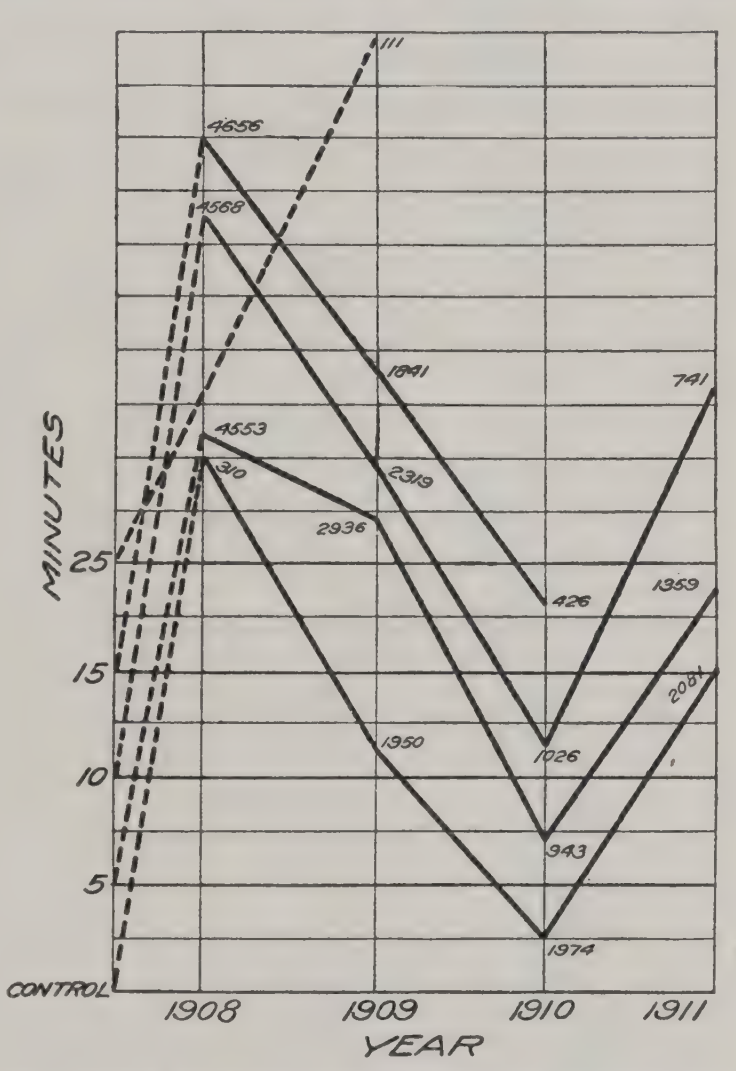



FIG. 5.—Curves summarizing for different years the percentages of infection in plantings of Red Amber sorgo after modified hot-water treatments at all temperatures and of all presoakings, but after treatments of various durations.

It is evident from the curves in all these illustrations not only that the treatments in no way reduced the amount of infection, but also that, regardless of treatment, the percentage of smutted plants occurring varied consistently with the season. Indeed, the curves in figures 4, 5, and 6 proved to be, with scarcely an exception,[1] so nearly alike for all the treatments that they could not well be drawn to the same coordinates. They are therefore separated, and each curve is continued by a broken line to the axis of the coordinates to which it is drawn, each interval therefrom representing 1 per cent of infection.

While it is true that infection by any phytopathogenic organism would vary with seasonal conditions regardless of the exact features of its life history, an added significance in these curves is found when it is noted that

[1] The only case in which these curves do not very nearly coincide is in the 54° C. treatments of 1909 (fig. 6). In this case there were but 151 heads on which to base the 1909 figure, this being so small that the result, which is characteristic of the irregular occurrence of the infection at Amarillo, is plainly dependent upon some peculiar minor factor, such as a variation in soil conditions, rather than upon the season. It is certainly not owing to the treatment of the seed.

the plantings at the Cereal Field Station at Amarillo were on new land both in 1907 and 1910. This station was established in 1907 and removed to another situation, also at Amarillo, at the latter date. In view of the fact that the presence of the organism has proved to be so salient a factor, as established by seed exchange and inoculation experiments, presented later, it would seem proper to attribute the light infection in 1907 and 1910 to the relative scarcity of the infective stage of the organism in the virgin soil. The large increase in 1908 was probably due to the proximity to the station of an old field which grew a rather badly smutted crop of sorgo each year. The decrease in 1909 was doubtless caused by drought, scarcely half of the crop being headed.

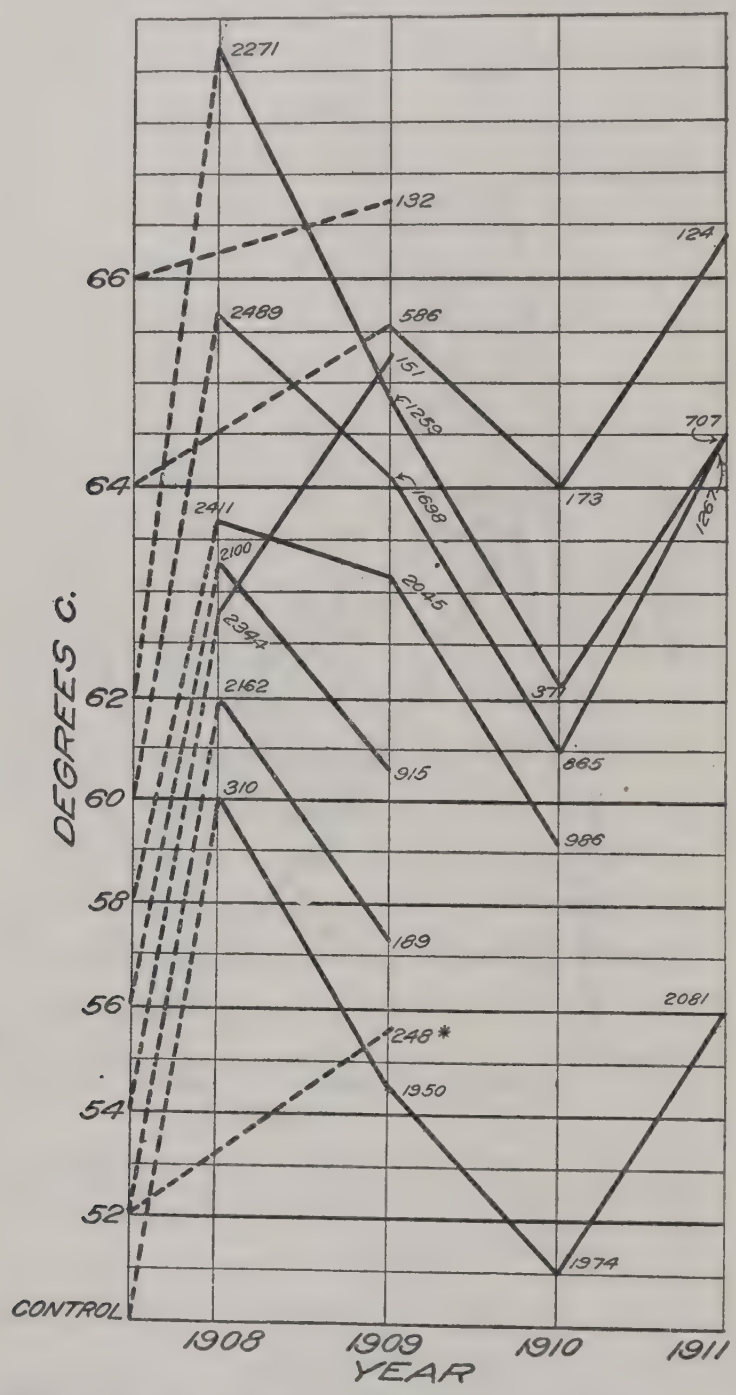



FIG. 6.—Curves summarizing for different years the percentages of infection in plantings of Red Amber sorgo after modified hot-water treatments of all durations of presoaking and treatment, but at various temperatures.

The inevitable conclusion from these experiments is that infection commonly takes place from some other source than seed-borne spores. This conclusion has been supported by tests of the effect of some of these treatments on the viability of the spores. Tables II and III present the results of these tests. They were somewhat obscured by the comparatively sparse germination so characteristic of these spores and by the development of the contaminations contained in the untreated spore material used in seeding check plates. The treatments with hot water were carried out, mostly on March 10, 1913, as follows.

Spores from Red Amber sorgo of the crop of 1911 were used in most cases. Before treatment they were thoroughly wet by shaking with distilled water. The dirt and foreign material were removed by centrifuging, and later the single spores were separated from the spore balls by the same method. In Table II, Nos. 1 to 14 and 29 to 34, inclusive, separated spores were used, while spore balls were used for the other treatments, except the last two, which were mixed. With a wire loop the spores or spore balls were transferred from the wet mass at the bottom of the centrifuge tube to tubes of water, which were then placed in the thermal bath. At the end of the period of treatment a portion of the spores in suspension was poured or pipetted out of the tube into melted agar at 43° C., in which they were shaken up and were then poured into a Petri dish. This portion was incubated at 27° to 28° C. and was examined from time to time under the microscope for germinating spores.

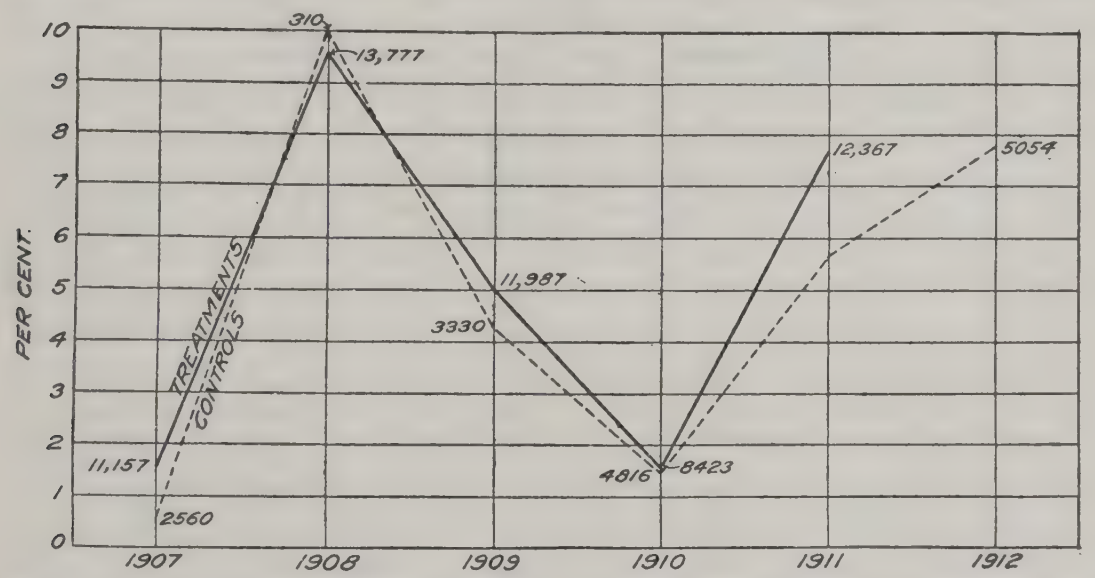



FIG. 7.—Curves summarizing for different years the percentages of infection: First, in plantings of sorgo after all seed treatments; and, second, in control plantings.

In the later treatments at 60° C. (Table II, Nos. 29 to 34, inclusive) the spores were subjected to the hot-water bath in the tubes of melted agar, thus avoiding the subsequent transfer. The first method would appear to give more chance for error, and to this is due, perhaps, the slight survival noted after rather severe treatments.

In Table II it is seen that moist heat is fatal within the upper range of temperatures used in the seed treatments (see fig. 6), and even dry heat seems injurious to the spores of this smut (Table II, Nos. 35 and 36). The plantings from hot-water and modified hot-water treatments of the seed showed a field infection in no way correlated with the thermal death point of the spores. About 24,000 plants grown from seed treated according to the latter method showed an infection of 5.9 per cent as against 3.1 per cent in about 3,500 plants grown from untreated seed. Over 15,000 of the plants from treated seed were of the Red Amber sorgo variety, which showed 6.5 per cent of smutted plants as against 4.2 per cent in the controls.

TABLE II.—*Results showing the effect of various hot-water and modified hot-water treatments on the viability of the spores of the head-smut organism*

| Serial No. | Treatment. | | | Duration of test. | Number counted. | Germination. |
|---|---|---|---|---|---|---|
| | Duration. | Temperature. | Duration of presoaking. | | | |
| | *Min.* | *° C.* | *Hours.* | *Days.* | | *Per cent.* |
| 1 | ..... | Control. | (a) | 2 | 1,225 | 3.8 |
| | | | | 3 | 535 | 4.5 |
| 2 | 10 | 55 | (a) | 3 | ..... | Trace. |
| | | | | 4 | ..... | Trace. |
| 3 | 20 | 55 | (a) | 3 | 1,500 | 0 |
| | | | | 4 | ..... | 0 |
| 4 | 10 | 60 | (a) | 3 | 3,000 | .1 |
| | | | | 5 | ..... | No increase. |
| 5 | 20 | 60 | (a) | 3 | 3,000 | 0 |
| | | | | 5 | ..... | 0 |
| 6 | 10 | 65 | (a) | 4 | 2,500 | .04 |
| 7 | 20 | 65 | (a) | 4 | 5,000 | 0 |
| 8 | ..... | Control. | 6 | 2 | 1,046 | 9.6 |
| 9 | 10 | 55 | 6 | 2 | 600 | 0 |
| | | | | 3 | ..... | 1— |
| | | | | 4 | 400 | 3 |
| 10 | 20 | 55 | 6 | 2 | ..... | 0 |
| | | | | 3 | ..... | Trace. |
| | | | | 4 | 700 | 2.9 |
| 11 | 10 | 60 | 8 | 3 | 3,000 | .03 |
| | | | | 5 | ..... | .5— |
| 12 | 20 | 60 | 8 | 3 | 4,000 | 0 |
| | | | | 5 | 5,000 | .02 |
| 13 | 10 | 65 | 8½ | 4 | 3,000 | .33 |
| 14 | 20 | 65 | 8½ | 4 | 5,000 | .04 |
| 15 | ..... | Control. | (a) | 2 | 572 | 1.6 |
| | | | | 3 | ..... | 5+ |
| | | | | 4 | ..... | No increase. |
| 16 | 10 | 55 | (a) | 3 | 400 | 0 |
| | | | | 4 | ..... | Trace. |
| 17 | 20 | 55 | (a) | 3 | 500 | 0 |
| | | | | 4 | ..... | 0 |
| 18 | 10 | 60 | (a) | 3 | 550 | 0 |
| | | | | 5 | ..... | 0 |
| 19 | 20 | 60 | (a) | 3 | ..... | 0 |
| | | | | 5 | ..... | Trace. |
| 20 | 10 | 65 | (a) | 4 | ..... | Trace. |
| 21 | 20 | 65 | (a) | 4 | ..... | Trace. |
| 22 | ..... | Control. | 6 | 2 | 503 | 19.7 |
| 23 | 10 | 55 | 6 | 2 | 402 | Slight. |
| | | | | 3 | ..... | 2+ |
| | | | | 4 | 100 | 30 |
| 24 | 20 | 55 | 6 | 2 | 650 | 0 |
| | | | | 3 | ..... | 0 |
| | | | | 4 | ..... | 0 |
| 25 | 10 | 60 | 8 | 3 | ..... | 0 |
| | | | | 5 | ..... | 0 |
| 26 | 20 | 60 | 8 | 3 | ..... | 0 |
| | | | | 5 | ..... | 0 |
| 27 | 10 | 65 | 8½ | 4 | 600 | .17 |
| 28 | 20 | 65 | 8½ | 4 | 522 | 0 |
| 29 | ..... | Control. | (a) | 2 | 1,000 | 4 |
| | | | | 8 | ..... | No increase. |
| 30 | 10 | 60 | (a) | 2 | ..... | 0 |
| | | | | 8 | ..... | 0 |

[a] Not soaked.

TABLE II.—*Results showing the effect of various hot-water and modified hot-water treatments on the viability of the spores of the head-smut organism*—Continued

| Serial No. | Treatment. | | | Duration of test. | Number counted. | Germination. |
|---|---|---|---|---|---|---|
| | Duration. | Temperature. | Duration of presoaking. | | | |
| | *Min.* | *° C.* | *Hours.* | *Days.* | | *Per cent.* |
| 31 | 20 | 60 | (a) | 2 | ..... | 0 |
| | | | | 8 | ..... | 0 |
| 32 | ..... | Control. | 6 | 2 | 950 | 4.5 |
| | | | | 8 | ..... | No increase. |
| 33 | 10 | 60 | 6 | 2 | ..... | 0 |
| | | | | 8 | ..... | 0 |
| 34 | 20 | 60 | 6 | 2 | ..... | 0 |
| | | | | 8 | ..... | 0 |
| 35 | | Control. | (a) | 3 | ..... | 13.1 |
| 36 | 5 | 70 Dry heat. | ..... | 2 | ..... | Slight. |
| | | | | 6 | ..... | Slight. |

[a] Not soaked.

In the tests of the effect of fungicides on the spores the solutions of different strengths, including water for control, were prepared at a temperature of 22° to 23° C. and placed in culture tubes. The spore material was prepared as for the thermal tests and transferred to the tubes in the same way. The culture tubes were then thoroughly shaken. At the end of the period indicated in the tables the tubes were again agitated and with a pipette 5 c. c. were removed from each to the centrifuge tubes, which were immediately filled with water. The spores being thrown down by centrifuging, the water was poured off and the tubes refilled, this rinsing being repeated four or five times. The last rinsing water from the strongest treatment was poured on to the control, which was then recentrifuged, to make certain that the rinsing had removed the treating solutions effectively. Further water being added, enough of the suspension of spores was poured into a tube of melted carrot agar at about 43° C. to make a thickly seeded plate. The plate was poured immediately, incubated, and examined as in the other tests.

In the work with copper sulphate, solutions equivalent to from 0.35 per cent to 2.52 per cent of $CuSO_4$ were used in treatments of sorgo seed, some of which had had the glumes removed before treatment. In one series (1907) a 17-hour soak with the weakest of these solutions gave plants with 2.3 per cent infection as against none in the controls, while in another series (1911), using seed without glumes, a 10-minute treatment with the strongest solution resulted in 13.1 per cent of infected plants as against 2.8 per cent in the controls.[1] Other treat-

[1] The fact that all of these treatment experiments, except the modified hot-water treatments, were also infected by *Sphacelotheca sorghi* seems to have had a peculiar bearing on these comparative percentages. In nearly all cases a considerably larger amount of head smut occurred in the treated lots than in the controls, which, not having been treated, were heavily infected by the kernel smut. The latter seemed to get the start of the head smut and prevent its development, for no case of evident double infection, as was observed by Busse (1904, p. 381), was found. Thus, in the various treatments of Red Amber sorgo carried out in 1911 with formalin, cresol, copper sulphate, and hot water, 24 treated lots containing 3,616 plants averaged 10 per cent of head-smut and 2.6 per cent of kernel-smut infection, while 15 lots (3,081 plants), untreated or unsuccessfully treated for the kernel smut, contained 5.8 per cent of head smut as against an infection of 29 per cent by the kernel smut. One lot with 62.3 per cent of kernel smut had 3.3 per cent of head smut; in another the percentages were 57.1 and 1.8, respectively. This phenomenon seems to have an adequate explanation in the comparatively late period of infection shown for the head smut (see p. 365).

ments involving, with controls, some 3,500 plants, were equally ineffective and inconsistent in their results.

But the spores are not at all injured by even more severe treatments. Table III, Nos. 11 to 18, inclusive, gives the results of these tests performed with the spores on March 7, 1913. It might even be said that the development of conidia proceeded better in the plates containing treated spores, probably on account of the absence of contaminations, these being for the most part killed by the treatment. It is possible that a longer treatment, even with less concentrated solutions, would have killed the spores (Herzberg, 1895, p. 30), but this would be likely to injure the seed as well.

TABLE III.—*Results showing the effect of various formalin and copper-sulphate treatments on the viability of the spores of the head-smut organism*

| Serial No. | Treatment. | | Duration of test. | Number counted. | Germination. |
|---|---|---|---|---|---|
| | Duration. | Method. | | | |
| | *Min.* | | *Days.* | | *Per cent.* |
| 1 | ..... | Control, not treated.... | 3 | 681 | 4.1 |
| | | | 7 | ..... | No increase. |
| 2 | 34 | 0.16 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | 1,000 | 1.6 |
| 3 | 33 | 0.24 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | ..... | 0 |
| 4 | 60 | 0.16 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | ..... | 0 |
| 5 | 60 | 0.24 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | ..... | 0 |
| 6 | ..... | Control, not treated.... | 3 | 541 | 13.1 |
| | | | 7 | ..... | No increase. |
| 7 | 60 | 0.16 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | ..... | Trace(?). |
| 8 | ..... | Control, not treated.... | 3 | ..... | Slight. |
| | | | 7 | ..... | Slight. |
| 9 | 60 | 0.16 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | ..... | 0 |
| 10 | 60 | 0.24 per cent formaldehyde solution ..... | 3 | ..... | 0 |
| | | | 7 | ..... | 0 |
| 11 | ..... | Control, soaked 30 minutes ..... | 28 | 1,068 | 5.2 |
| 12 | 30 | 0.76 per cent copper-sulphate[a] solution... | 28 | 1,044 | 5.0 |
| 13 | 30 | 1.52 per cent copper-sulphate solution.... | 28 | 1,034 | 5.2 |
| 14 | 30 | 2.52 per cent copper-sulphate solution.... | 28 | 1,000 | 4.0 |
| 15 | ..... | Control, soaked 60 minutes ..... | 28 | 1,034 | 4.5 |
| 16 | 60 | 0.76 per cent copper-sulphate solution.... | 28 | 1,000 | 1.8 |
| 17 | 60 | 1.52 per cent copper-sulphate solution.... | 28 | 1,000 | 5.0 |
| 18 | 60 | 2.52 per cent copper-sulphate solution.... | 28 | 1,000 | 6.0 |

[a] Copper sulphate = $CuSO_4$.

In an additional test intended to discover the influence of a residual effect of the fungicide after treatment without rinsing, it was found that the presence of a trace of copper sulphate in the medium does not hinder germination. However, Moore and Kellerman (1904, p. 29) found that the toxic action of dilute watery solutions of copper is overcome by certain substances present in most culture media; and Hawkins (1913, p. 68–75) has recently shown that soluble calcium and potassium salts also neutralize the toxicity of copper. The probability that some of these substances were present in the vegetable medium used makes the above test of residual action inconclusive. Nevertheless, Dandeno (1908, p. 60) states that *Ustilago zeae* germinates readily in a N/2,048 watery solution of copper sulphate. Copper fungicides do not appear to have a very penetrating action, and the sulphate certainly is not destructive to the head-smut spores within a limited time at ordinary temperatures.

McAlpine (1910, p. 298) found that a 0.12 per cent solution of formaldehyde did not affect the spores inside of 15 minutes. However, the formaldehyde treatment, when sufficiently severe, does kill them, as is shown in Nos. 1 to 10, inclusive, of Table III. These tests were with separate spores, except in the last three, in which spore balls were used.

In spite of this evidence that the spores do not survive one hour's treatment with a 0.16 per cent formaldehyde solution, it was found that seed given this and more severe treatments produced plants with 4.2 per cent of infection in about 3,000 plants (the estimates in the early experiments were by heads) which survived, as against 3.4 per cent in about 2,000 plants from untreated seed. The formalin treatment, therefore, is ineffective, but not because of failure to destroy external seed infection; and it may be said that this is true of the other chemical treatments of the seed, all of which have proved equally ineffective in prevention, even though, like copper sulphate, they may have had no lethal effect upon the spores. Indeed, plants from treated seed seemed the more easily infected in some instances.

## FLORAL INOCULATIONS

The evident systemic character of the disease, however, immediately suggested the possible analogy with the loose smuts of barley and wheat. Kellerman's inoculations were made before the possibility of intraseminal infection was realized, and the question occurs, was not Jensen's (1888, p. 61) mistake, in assuming extraseminal infection to have taken place in the case of *Ustilago tritici* when a diseased wheat plant appeared among those he had inoculated, here repeated in the case of sorghum? While the loose character of the spore body and the echinulate spores of the head smut gave added force to the hypothesis of a floral infection, the abundant production of conidia and, as compared with the loose smuts (Appel and Riehm, 1911, p. 363), the prolonged viability of the spores, did not support this analogy.

Numerous floral inoculations, undertaken early in these investigations, also failed to give results supporting this view. These were carried out in several different seasons and at various stages of development in the ovary. Dry spores of the head smut were placed in a paper bag and shaken into the flowers by inverting the bag over a head and shaking thoroughly; sometimes they were placed inside the glumes with a camel's-hair brush. Some of the spores were germinated before applying them, and were sprayed into the flowers with an atomizer either by opening the glumes with forceps, or in the early morning while the plant was still in bloom; some of the heads were not covered, but some were kept covered for a time with paper bags or with a large lamp chimney to keep them moist. This was an extremely difficult matter, however, owing to the high winds and to the consequent rapid rate of evaporation, which, from an open water surface, often exceeds half an inch in 24 hours at Amarillo. While there was occasionally a rather high percentage of infection in the resulting plants, this was not the uniform result of any particular method of inoculaton; nor was it sufficiently large to obviate its explanation by infection of the plants during development in the field, in view of the fact that it did not occur consistently.

## ENVIRONMENTAL EXPERIMENTS

In addition to the negative results of inoculations, it was found that seed from the same lots when planted at various points in the United States, or in different seasons at Amarillo, gave very different amounts of infection in the plants produced, while in plants from different lots of seed, grown at the same station, no consistent differences could be observed.

A preliminary experiment was carried out in 1908. The plants were all grown from the same lot of seed, yet those grown at Amarillo were 7.7 per cent smutted and those at Chillicothe, Tex., were 2 per cent smutted, while those at McPherson, Kans., were not affected at all. In 1910 a new series was begun. Plantings were made from two lots of seed at eight different stations, including Amarillo and Chillicothe, Tex., St. Paul, Minn., and Arlington, Va. Of these two lots, that from Chillicothe happened to develop the greater percentage of smutted plants at Amarillo, and the seed grown from it was therefore used for the plantings in 1911. In this and subsequent seasons the intention was to plant at each station seed from each of the places concerned and to use only seed descended from the original lot and grown in consecutive seasons at the same station. This was usually done, but, owing to various mishaps, the plantings at the four stations named were the only ones which were carried completely through the experiment as intended. The data from these four stations thus form a complete series and are summarized in Table IV. They involved in each case from about 150 to 800 plants; usually about 300.

TABLE IV.—*Summary of results showing the influence of locality on the occurrence of head smut*

| Seed from— | Percentage of infection at— | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amarillo, Tex. | | | Chillicothe, Tex. | | | St. Paul, Minn. | | | Arlington, Va. | | |
| | 1910 | 1911 | 1912 | 1910 | 1911 | 1912 | 1910 | 1911 | 1912 | 1910 | 1911 | 1912 |
| Amarillo, Tex | 1.6 | 14.71 | 10.86 | 0.14 | 0 | 7.34 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chillicothe, Tex | 3 | 13.09 | 12.29 | .25 | 0 | 6.93 | 0 | 0 | 0 | 0 | 0 | 0 |
| St. Paul, Minn | ..... | 17.14 | 1.72 | ..... | .87 | 3.52 | ..... | 0 | 0 | ..... | 0 | 0 |
| Arlington, Va | ..... | 7.56 | 2.9 | ..... | 2.6 | 4.46 | ..... | 0 | 0 | ..... | 0 | 0 |

From this it may be seen that no infection occurred at Arlington or at St. Paul. Only a trace of it has ever occurred at St. Paul, except in inoculated plants in 1912. It has not been present at all at the Arlington Experimental Farm or in its immediate vicinity during the three years in question, so far as the writer was able to discover by careful examination. Yet seed from St. Paul produced the highest percentage recorded at Amarillo in 1911, although showing no infection at either Arlington or St. Paul in that year; and seed from Arlington has always produced some smutted plants at the two Texas points. Of the four seed lots used in 1911, the Arlington seed produced the largest number of infected plants at Chillicothe. Moreover, seed grown at either of the two Texas stations never produced smutted plants when grown at the other two stations, although inoculated plants showed abundant infection at St. Paul in 1912 (see Table V, plat E). It should be noted, too, that seed from the same lots used for the Amarillo plantings in 1910 and 1911 were planted at Amarillo in the ensuing years and produced infected plants as follows: 1910 lots, replanted in 1911, 3.8 per cent and 15.6 per cent, respectively; 1911 lots, replanted in 1912, 1.8, 2.7, 0, and 1.8 per cent, respectively. These figures are evidently in no way comparable or consistent with those of the year before, as shown in Table IV.

## EXPERIMENTS WITH PROTECTED SEED

As may have been already observed, particularly in connection with the slight irregularities of the curves in figure 6 (see footnote, p. 351), positive conclusions from comparative amounts of infection in small lots of plants at Amarillo are not warranted without consistent results from oft-repeated experiments. However, the appearance of any infection in plants from seed protected from contamination gives additional evidence that the infection is not carried with the seed.

Thus, 177 plants were grown at Amarillo in 1912 from seed produced in the greenhouse at Washington, D. C., on heads which had been covered with transparent paper bags from before flowering until they were thrashed out by hand. One plant (0.6 per cent) was infected. Similarly, 1,669 plants grown in 1912 from seed of 18 heads protected in the

same way but produced in the field at Amarillo in 1911 showed 6.4 per cent of infection. The high winds had torn some of the bags at times, but they were replaced as soon as possible. Moreover, four of them remained intact throughout; yet of the 206 plants grown from the resulting seed, 13, or 6.3 per cent, were infected. This was scarcely less than the average natural field infection in 1912. (See fig. 7.)

This evidence is a particularly strong negation of the floral infection theory, especially when it is noted that the seed lot from the greenhouse in Washington, D. C., produced 8 infected plants out of 18 when the seedlings were artificially inoculated. (See Table V, plat C, No. 5.)

## INFECTION EXPERIMENTS

It has been made clear by the results already described that floral infection is not involved in the life history of this parasite and that seed-borne spores, though doubtless functioning at times in distributing the fungus from one district to another, by no means constitute the determining factor in the general field infection. The apparent contradiction in the evidence so far presented—one which has led to many confusing surmises and recommendations in the literature of the subject—remains to be explained by positive evidence of infection from artificial inoculations.

A series of inoculation experiments carried on at Amarillo, Tex., in 1911, duplicated at Amarillo, Tex., at St. Paul, Minn., and at Manhattan, Kans., during the season of 1912, and twice repeated in the greenhouse at Washington, D. C., has confirmed these observations and demonstrated that the presence of the parasite in the soil about the growing seedling is productive of successful infection under any of the conditions prevailing in these various habitats. These results are presented in Table V.

### EXPLANATION OF TABLE V

In tabulating these results considerable abbreviation has seemed desirable, and it is herewith explained. When special reference to this explanation is necessary, the abbreviations in Table V are inclosed in parentheses. Under each of the following main headings the column with the same heading in the table is explained.

"Date."—The date given in the column provided is the date of inoculation except in a few cases, usually controls, when it is inclosed in parentheses and indicates the date of planting.

"Seed Lot."—Five different lots of seed, all of the variety Red Amber sorgo (S. P. I. No. 17548), were used and are indicated, in the column provided, by the following symbols:

"I." From the crop of 1910 at Amarillo, Tex. When in parentheses, as "(I)," the seed had the glumes still inclosing it; otherwise it was without them, having been separated in water from the seed which had retained the glumes through the thrashing process.

"II." Seed without glumes (separated in water, as in I); from the crop of 1911 at Amarillo. This seed was treated with a 0.24 per cent formaldehyde solution for one hour, except where the symbol is in parentheses "(II)."

"III." Seed from a head grown at Amarillo in 1911, which had been kept covered with a transparent paper bag from before flowering until thrashed out by hand. The parentheses simply indicate a different head as the source of seed.

"IV." Seed from heads grown in the greenhouse at Washington, D. C., during the winter of 1911–12 and kept covered, as above, from before flowering until thrashed out by hand.

"V." Seed without glumes (separated in water, as in I); from the crop of 1911 at Akron, Colo. Treated with 0.16 per cent formaldehyde solution for 10 minutes after a thorough washing.

"Spore Lot."—The mixed lot of spores used is so indicated; the other five lots, all collected from Red Amber sorgo at Amarillo, are indicated as follows:

"*A*." Collected in the fall of 1910.

"*B*." Collected in September, 1911. The parentheses indicate conidia from cultures first isolated from single spores of this lot (see p. 341) in February, 1912.

"*C*." Collected in the fall of 1912.

"Method."—The methods used in making inoculations are classified—

First, as to the condition of the host plant when inoculated (or planted, in the controls):

"a"=dry seed;

"b"=germinating seed;

"c"=older plants.

Second, as to the character of the inoculating material:

"m"=dry spores;

"n"=suspension of spores in which a few were germinating;

"p"=conidia.

Third, as to the general procedure in inoculating:

"x"=heavy application of a mass of the inoculating material, usually so as to completely cover the seed or seedling when planting it, or, on older plants, to cover the inoculated part;

"y"=lighter application—dusting of dry spores before planting or spray of material in water;

"z"=inoculation of the plat by raking smutted heads into the soil after plowing in the spring. "zz" in plat C, No. 7=inoculation two years in succession, the same plat being used as for plat A, No. 11, the year before.

Fourth, the controls, which were not artificially inoculated, are indicated in this column.

Fifth, special methods in inoculation are indicated by parentheses, as follows:

"bm(x)" in plat A, No. 7=the soil in the opened row was heavily inoculated at planting;

"bn(x)" in plat B, Nos. 1 and 2, plat D, No. 1, and plat E, No. 1, and

"bp(x)" in plat C, Nos. 1 and 2, plat D, No. 2, and plat E, No. 2=both seedling and soil were inoculated;

"cm(x)" in plat C, No. 8, plat D, No. 8, and plat E, No. 5=the spores were placed about the root crown just beneath the surface of the soil;

"cp(x)" in plat E, No. 6=the conidia were taken from carrot-agar culture and smeared on the base of the plant with a flat inoculating needle;

"bm(y)," "bn(y)," and "b(control)" in plat E, Nos. 8, 9, and 10=the ground was thoroughly wet down both before and after planting, the seed only being inoculated;

"bn(y)" in plat A, Nos. 1 and 2=the seed only was inoculated;

"bn(y)" in plat A, Nos. 3 and 4=the soil only in the opened row was inoculated;

"cp(y)" in plat C, No. 9, and plat D, No. 9=conidia were sprayed on the root crown, which was then re-covered with moist earth.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*

PLAT A[a]

[Planted at Amarillo, Tex., in the field; counted Sept. 12, 1911.]

| Serial No. | Date. | Seed lot. | Spore lot. | Method. | Total number of plants. | Infection. |
|---|---|---|---|---|---|---|
| | | | | | | *Per cent.* |
| 1 | May 25 | I | Mixed | bn(y) | 325 | 4.9 |
| 2 | do | (I) | do | bn(y) | 383 | 3.7 |
| 3 | do | I | do | bn(y) | 103 | 5.8 |
| 4 | do | (I) | do | bn(y) | 70 | 5.7 |
| 5 | do | I | do | bmy | 165 | 6.7 |
| 6 | do | (I) | do | bmy | 210 | 5.3 |
| 7 | do | I | do | bm(x) | 130 | 34.6 |
| 8 | May 26 | (I) | do | bm(x) | 106 | 23.6 |
| 9 | May 25 | I | do | amy | 200 | 10 |
| 10 | do | (I) | do | amy | 292 | 4.1 |
| 11 | (May 23) | I | *A* | az | 34 | 11.7 |
| 12 | do | (I) | *A* | az | 110 | 5.5 |
| 13 | May 25 | I | .. | a, control[b] | 196 | 6.6 |
| 14 | do | (I) | .. | a, control | 127 | 12.6 |
| 15 | do | I | .. | a, control | 272 | 7.3 |
| 16 | do | (I) | .. | a, control | 444 | 3.8 |

PLAT B[a]

[Planted at Washington, D. C., in pots in the greenhouse of the Department of Agriculture. The even numbers were planted in a 2-inch top dressing of clean sand, while the other pots (odd numbers) contained only potting soil; counted May 16, 1912.]

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | (c) | II | *B* | bn(x) | 8 | 50.0 |
| 2 | (c) | II | *B* | bn(x) | 7 | 14.3 |
| 3[d] | (c) | II | *B* | bmx | 3 | 100 |
| 4 | (c) | II | *B* | bmx | 3 | 100 |
| 5 | (c) | II | .. | a, control | 5 | 0 |
| 6 | (c) | II | .. | a, control | 7 | 0 |
| 7 | (c) | (II) | .. | a, control | 7 | 0 |
| 8[d] | (c) | (II) | .. | a, control | 8 | 0 |
| 9 | (e) | II | *B* | bmx | 2 | 50 |
| 10 | (e) | II | *B* | bmx | 1 | 100 |

PLAT C[f]

[Planted at Amarillo, Tex., in the field; counted Sept. 7, 1912.]

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | May 28 | II | (*B*) | bp(x) 0.6 to 1.2[g] | 41 | 26.8 |
| 2 | May 30 | III | (*B*) | bp(x) | 5 | 0 |
| 3 | May 28 | II | *B* | bmx 0.6 to 1.2[g] | 45 | 66.6 |
| 4 | May 30 | (III) | *B* | bmx | 1 | 0 |
| 5 | May 28 | IV | *B* | bmx | 18 | 44.4 |
| 6 | do | II | *B* | amx | 102 | 42.2 |
| 7 | (May 29) | II | *B* | azz | 522 | 21.45 |

[a] Inoculations by the author.
[b] Treated for one hour with 0.24 per cent formaldehyde solution.
[c] About Nov. 15, being the date of planting in Nos. 5, 6, 7, and 8.
[d] See Plate XXXV, figure 3.
[e] About Feb. 8.
[f] Inoculations performed by Mr. E. C. Johnson.
[g] The numbers given indicate in centimeters the length of the plumules in Nos. 1 and 3, and the average height of the plants in Nos. 8 and 9. In the latter case the plants were mostly unbranched as yet. In Nos. 10 and 11 the plants were younger than in No. 3.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*—Continued

PLAT C—Continued

| Serial No. | Date. | Seed lot. | Spore lot. | Method. | Total number of plants. | Infection. |
|---|---|---|---|---|---|---|
| | | | | | | *Per cent.* |
| 8 | June 25 | II | *B* | cm(x)5[a] | 136 | 2.94 |
| 9 | do | II | (*B*) | cp(y)5[a] | 112 | 7.1 |
| 10 | May 29 | II | *B* | bmx[a] | 45 | 42.2 |
| 11 | do | II | *B*[b] | bmx[a] | 68 | 39.7 |
| 12 | (May 29) | II | .. | b, control | 112 | 32.1 |
| 13 | do | II | .. | a, control | 148 | 1.4 |
| 14 | do | II | .. | a, control | 185 | 3.2 |
| 15 | do | (II) | .. | a, control | 136 | 25 |
| 16 | do | (II) | .. | a, control | 114 | 3.5 |
| 17 | (May 25) | (III) | .. | a, control | 51 | 3.9 |
| 18 | do | III | .. | a, control | 50 | 4 |
| 19 | (May 29) | II | .. | a, control[c] | 328 | 1.5 |
| 20 | do | (II) | .. | a, control[c] | 268 | 0 |

PLAT D[d]

[Planted at Manhattan, Kans., in the field; counted Aug. 30, 1912.]

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | June 4 | II | *B* | bn(x)2[e] | 1 | 0 |
| 2 | do | II | (*B*) | bp(x)2[e] | 24 | 0 |
| 3 | June 3 | II | (*B*) | bp(x)5[e] | 1 | 0 |
| 4 | June 4 | II | *B* | bmx2[e] | 24 | 29.1 |
| 5 | June 3 | II | *B* | bmx3[e] | 3 | 0 |
| 6 | do | II | *B* | bmx5[e] | 1 | 100 |
| 7 | June 3–4 | II | *B* | amx | 50 | 10 |
| 8 | June 4 | II | *B* | cm(x) | 200 | 0 |
| 9 | do | II | (*B*) | cp(y)[c] | 75 | 0 |
| 10 | (June 4) | II | .. | a, control | 210 | 0 |
| 11 | do | II | .. | a, control[c] | 731 | 0 |
| 12 | do | II | .. | b6, control[e] | 5 | 0 |
| 13 | do | II | .. | b2, control[e] | 26 | 0 |

PLAT E[f]

[Planted at St. Paul, Minn., in the field; counted about Sept. 20, 1912.]

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | June 7 | II | *B* | bn (x) | 49 | 10.2 |
| 2 | do | II | (*B*) | bp (x) | (*g*) | 0 |
| 3 | do | II | *B* | bmx | 30 | 26.7 |
| 4 | do | II | *B* | amx | 49 | 36.7 |
| 5 | July 5 | II | *B* | cm (x)[h] | (*g*) | 0 |
| 6 | do | II | (*B*) | cp (x) | (*g*) | 0 |
| 7 | June 8 | II | .. | a, control | (*g*) | 0 |
| 8 | June 11 | II | *B* | bm (y) 2.5[h] | 85 | 24.7 |
| 9 | do | II | *B* | bn (y) 2.5[h] | 98 | 2 |
| 10 | (June 11) | II | .. | b2.5 (control)[h] | (*g*) | 0 |

[a] The numbers given indicate in centimeters the length of the plumules in Nos. 1 and 3, and the average height of the plants in Nos. 8 and 9. In the latter case the plants were mostly unbranched as yet. In Nos. 10 and 11 the plant were younger than in No. 3.

[b] Kept outside in cloth bag through the winter at Amarillo.

[c] Planted apart from the rest to avoid contamination from inoculated rows.

[d] Inoculations by the author, assisted by Dr. N. E. Stevens.

[e] These numbers indicate the time, in days, between setting the seed to germinate and inoculating and planting it.

[f] Inoculations performed by Dr. E. M. Freeman and Mr. J. H. Parker.

[g] Plants not counted.

[h] This number indicates the approximate length of the plumules in centimeters at the time of inoculation and planting.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*—Continued

PLAT F [a]

[Planted at Washington, D. C., in the greenhouse in beds separated by partitions 1 foot deep in the soil; counted Apr. 3, 1913.]

| Serial No. | Date. | Seed lot. | Spore lot. | Method. | Total number of plants. | Infection. |
|---|---|---|---|---|---|---|
| | | | | | | *Per cent.* |
| 1 | Nov. 1–9 | V | *B* and *C* | Various [b] | 67 | 55.2 |
| 2 | do | V | .. | a, control | 16 | 0 |
| 2 [c] | do | V | .. | do | 16 | 31.25 |

[a] Inoculations by the author.

[b] These inoculations were performed with various methods and stages of growth in an effort to get more exact information. With the small number of plants, necessitated by the use of a greenhouse, differences in the amount of infection appearing were of little significance in view of the impossibility of properly controlling conditions. Most of the plants were not directly watered, except at planting (nor were they, in the control), until mature in the spring. Although all were grown in separate beds instead of pots and obtained ample moisture from below, they were much stunted by greenhouse conditions.

[c] The same plants as above, but counted Oct. 3, 1913.

While most of the results of these inoculations are positive beyond a doubt, an important negative result, as yet unexplained, should be pointed out. The conidia, in spite of the care taken to be certain of their identity (see p. 341), have never produced the slightest evidence of infective power in the few trials made in the field (plat C, Nos. 1, 2, and 9; plat D, Nos. 2, 3, and 9; and plat E, Nos. 2 and 6). Brefeld (1895, p. 30) has found that oat smut, like many other pathogenic organisms, loses its virulence after several months in artificial cultures. Unless *Sorosporium reilianum*, as cultivated on carrot agar in these investigations, lost its infective power very quickly, however, this explanation does not seem adequate, for new cultures grown artificially for only two or three weeks produced no infection when inoculated on 15 plants at the same time and under the same conditions as plat F, No. 1. The conidia have not been observed to produce infection threads as figured by Brefeld (1883, pl. 11, fig. 7).

The first question which arises on considering the fact, here now clearly shown, that extraseminal infection does take place, is, What factor has been introduced to bring about successful infection when so many former attempts had failed? The results given in Table V, while not exhaustive, do make clear several of the essential points in the parasite's life history which will at least partially answer this question. The method designated under the abbreviation bmx will be observed to have produced the most consistently positive results wherever tried. Except at Amarillo, the only other methods which produced over 20 per cent infection were bn(x) in pots in the greenhouses of the Department (plat B, Nos. 1 and 2 of Table V), amx at St. Paul (plat E, No. 4), and bm(y) at St. Paul (plat E, No. 8), besides the inoculations later attempted in the greenhouse (plat F).

Since none of these methods can be presumed to correspond closely to the natural process of infection, the conclusions drawn from them must be largely a matter of inference. The small number of plants and the abnormal conditions in the greenhouse make it unnecessary to consider method bn(x), in plat B, Nos. 1 and 2, further than to note that both seeds and soil were heavily inoculated and that the seeds were germinating. Moreover, method bmx in the same series (plat B, Nos. 3 and 4) produced 100 per cent of infection on six plants, so that both these methods appear to have been proportionately more successful than elsewhere, probably because of the more thorough technique where so few plants were concerned. It appears, indeed, that the abundance of infectious material provided has been the most salient factor involved. Without it at Amarillo natural infections were often so numerous that the effect of inoculation was not perceptible; compare, for instance, plat A, No. 7, with plat A, Nos. 9 and 13, and plat A, No. 8, with plat A, Nos. 10 and 14. Method amx, which is closely similar to bmx on account of the large amount of spore material provided, the seedling having to grow up through the latter in both cases, has also produced a comparatively large percentage of infection, even exceeding bmx (plat E, No. 3) at St. Paul.

These results immediately suggest that no such crucial period for infection of the seedling obtains in the case of this smut as has been observed by Brefeld (1895, p. 46) for *Ustilago cruenta*, for the presence of the infecting organism during the whole of the early development of the host produces the disease when its presence on the seed alone will rarely do so. While *U. cruenta* was not able to infect, in Brefeld's experiments, after the leaf sheath had been split as far down as 1 cm. from the tip, the plumules of the plants inoculated by method bm(y)—a dusting of dry spores over the seedlings—in plat E, No. 8, averaged close to 2.5 cm. in length and yet were nearly as abundantly infected as those which were smaller and more heavily inoculated four days before (plat E, No. 3).

The difference between the latter and plat E, No. 4 (method amx) is not sufficient to militate against the conclusion that a late period of infection is possible, although it has seemed from the character of the infection in the mature plant, as revealed by the histological studies already discussed, that the infection in the field at Amarillo is usually quite early in its origin. That it is systemic in the individual culm more characteristically than in the plant as a whole, however, supports this idea of late infection (see p. 348). Investigation has shown, moreover, that the hyphæ were at least not widely disseminated in the growing tissues of several seedlings which later developed infection. In the seasons of 1910[1] and 1911 about 200 seedlings at three to four weeks after planting were dissected and a part of the meristem—that containing the primary

[1] The dissections in this season were made by Mr. V. L. Cory.

growing point—was removed and preserved. The plants were then induced to produce a second growth from what remained. The meristem of those which developed head smut at maturity was then carefully examined; yet in none of the 16 plants which developed the disease could the hyphæ be found in the parts preserved.

In additon to the negative evidence of these dissections, Mr. Karl F. Kellerman, of the Bureau of Plant Industry, stated to the writer in recent conversation that he performed a number of experiments with this smut by artificial inoculations on sorghum in the greenhouse while working in Ohio with his father, Dr. W. A. Kellerman. The plants were in pots and were inoculated at stages varying from the time they first appeared above ground until they were about 5 inches high. The method used was to wash the soil away from the roots, sift dry spores over them, and re-cover with soil. While some indications pointed to infection through the roots, this was not definitely established. Whatever the mode of entry, however, the parasite proved able under the conditions in the greenhouse to infect plants at all the stages at which they were inoculated.

In the recent greenhouse experiments (Table V, plat F, No. 1) some of the plants were successfully inoculated after the first leaf had begun to turn green. But, most unexpected of all, after leaving these plants to grow all summer it was found in October that the control (plat F, No. 2) contained five smutted plants, whereas the original culms which developed in April showed no sign of the disease. Other plants, too, which had not been smutted in the spring had grown smutted culms by fall. While Hecke (1907, p. 572) has presented similar facts as proof of shoot or branch ("Trieb") infection by *Ustilago antherarum*, in the case of sorghum, at least, there is some uncertainty as to the exact point of infection. The inoculation of the nodal buds has been tried a few times in the greenhouse without result. This does not preclude the possibility of such an infection, however, and more careful work supported by histological observations is needed.

It does not seem that the spread of the disease from plant to plant under greenhouse conditions makes it probable that such an occurrence is at all common in the field, but it does add certainty to the conclusion that infection by this smut is by no means confined to the early seedling stage of the host. This, then, together with the sparse germination of the spores, readily explains the repeated failures to produce any appreciable amount of infection by inoculation of the seed.

In Table V, plats C, D, and E, it will be observed that the same lot of seed, "Seed lot II," previously treated with a 0.24 per cent solution of formaldehyde, was used for nearly all the inoculations. This seed produced plants free from head smut at both Manhattan, Kans., and St. Paul, Minn. (plats D, Nos. 10, 11, 12, and 13, and E, Nos. 7 and 10), except when artificially inoculated; but at Amarillo all but one of the

control plantings (plat C, Nos. 12 to 20, inclusive) were infected—two of them to the extent of 25 per cent or more—while the percentage of infection in the successful inoculations was not remarkably greater, as compared with controls, than was produced by the same methods at the other two stations. It is thus indicated that at Amarillo, or wherever this smut occurs at all commonly, the parasite is present, doubtless in the soil, in much the same way as the common maize smut, *Ustilago zeae*, is present where maize is much grown.

## PREVENTION OF HEAD SMUT

Since the period of infection appears to be quite indefinite, the prevention of this disease seems almost as difficult a problem as that of dealing with common maize smut, and, where prevalent, is a more serious question on account of the more systematic character of the infection. This latter fact, however, suggests a possible, though very doubtful and as yet untried, specific measure for prevention—i. e., the treatment of the soil about the seed at planting time in some such way as is done for onion smut—in the hope of keeping infection away from such buds as develop early in the life of the plant.

The fact that the disease occurs most abundantly in a district where manures or fertilizers have rarely, if ever, been used obviates the explanation of its occurrence on this basis. The Panhandle of Texas is, however, a region of high winds favorable to its spread, and the cutting out and burning of the whole plant when one is found infected should, of course, be recommended. Rotations planned to avoid continuous cropping of the particularly susceptible sorgo varieties on the same ground or to the leeward of prevailing winds from such a field should also considerably reduce the amount of head smut.

An important element in the relation of the problem to the grain-sorghum grower is the fact that milo, as has been noted by Freeman and Umberger (1908), is a variety apparently immune from all the sorghum smuts. This crop is widely grown in the southern part of the Great Plains, and it should be possible, theoretically, to obtain various immune varieties adapted to other sections by breeding from it. Since the cause of this immunity is not yet apparent, however, it can not be definitely stated that its hybrids will partake of this character. Kafir and broom corn, while much less susceptible to this smut than the sorgos, are quite subject to the attack of the kernel smut. This lack of immunity might prove serious to these crops or even to maize, should the head smut ever become as abundant as has maize smut (*Ustilago zeae*) in many sections. The latter is indigenous to America, however, and since the head smut is not, it may be hoped that adequate quarantine measures would prevent its spread and lead, perhaps, to its final eradication.

SUMMARY

(1) The head smut of sorghum, *Sorosporium reilianum* (Kühn) McAlpine, was first reported from Egypt in 1868. It has been found to be a destructive parasite, though not yet of widespread occurrence in this country. It occurs also on maize, or Indian corn.

(2) The organism has been grown in artificial culture. Its growth is almost exclusively conidial under favorable conditions, the optimum temperature being 28° to 30° C. As with several others of the Ustilagineae, spore-like bodies are occasionally found in older cultures.

(3) Although perfect sori of the parasite are not usually produced in every head of a plant, most of the stools and branches are so affected, even when producing no spore-bearing tissue, that the inflorescence is sterile and often peculiarly proliferated. This vegetative stimulus results also in the development of the lateral buds into branches.

(4) Histological studies indicate an early period of infection and the systemic nature of the disease. The lateral buds carry the infection in their meristematic tissue apparently from the time of their formation when the culm is starting to differentiate the nodes.

(5) The work of other investigators, though not conclusive, pointed to infection from seed-borne spores and the possibility of applying the usual seed-treatment methods for preventing the disease. Both of these contentions have been shown to be untenable by an extensive series of ecological experiments and exhaustive tests of various sterilizing agents, including the use of thermal methods, on the seed.

(6) Numerous floral inoculations failed to show that the infection was produced intraseminally and carried over in the seed to the next crop. On the other hand large percentages of infection were repeatedly produced by inoculation of the seedlings with dry spore material, some becoming infected in the greenhouse even after the first leaf had emerged from the sheath and begun to turn green. While the process of infection has not yet been observed histologically, it is clearly proved that the parasite is not carried with the seed, but is wind-distributed in the locality in which it occurs, doubtless infecting the seedling from the soil.

(7) Though widely distributed in the tropical and semitropical countries of the world, the head smut has been known in this country for only about 35 years. Methods of combating it are especially needed in order to prevent its spread. Fortunately the widely grown variety, milo, has proved immune from all the smuts of sorghum.

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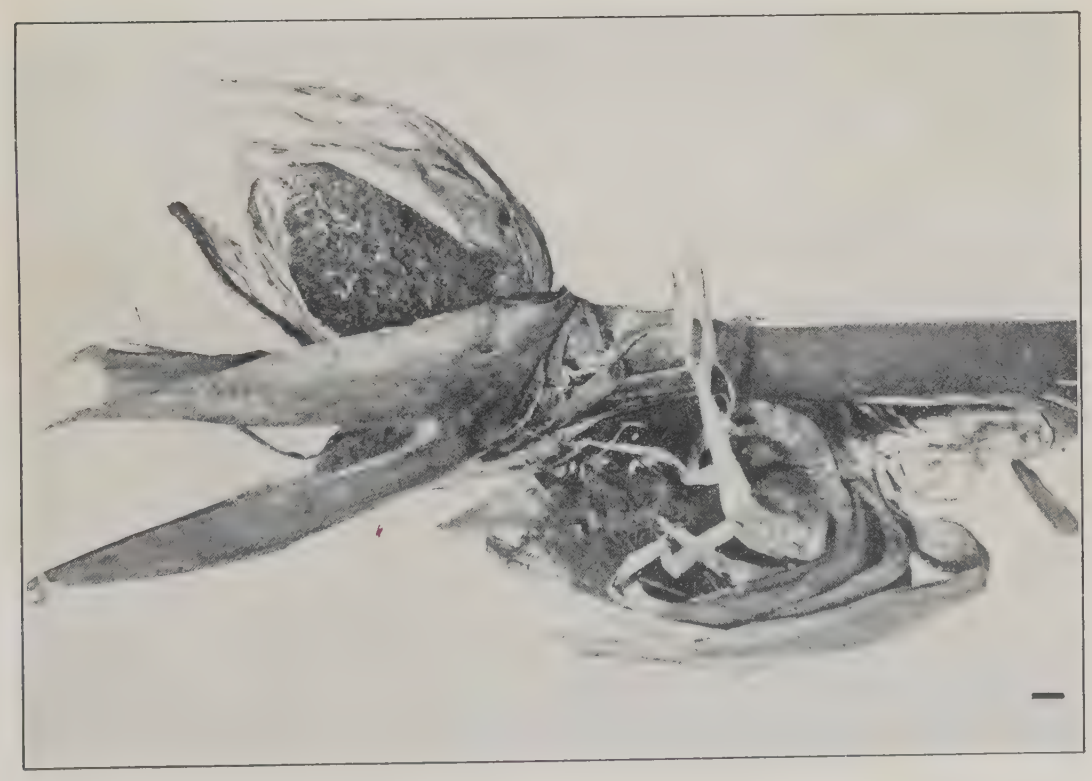
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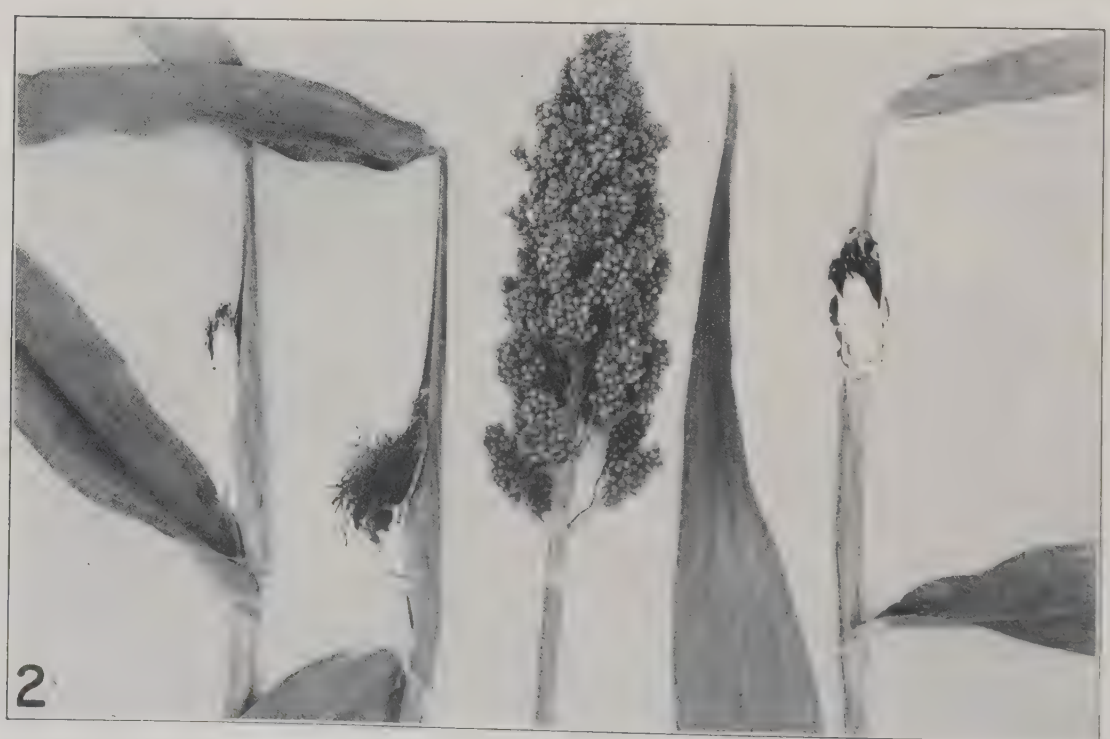
## PLATE XXXI

Fig. 1.—Head smut in ear of maize (after McAlpine).
Fig. 2.—Head smut in tassel of maize (after Evans).


2


1


a
b
c
1


2

## PLATE XXXII

Fig. 1.—The three American species of sorghum smut on Blackhull kafir: (a) *Sphacelotheca cruenta*, (b) *Sorosporium reilianum*, (c) *Sphacelotheca sorghi*. Photographed by author.

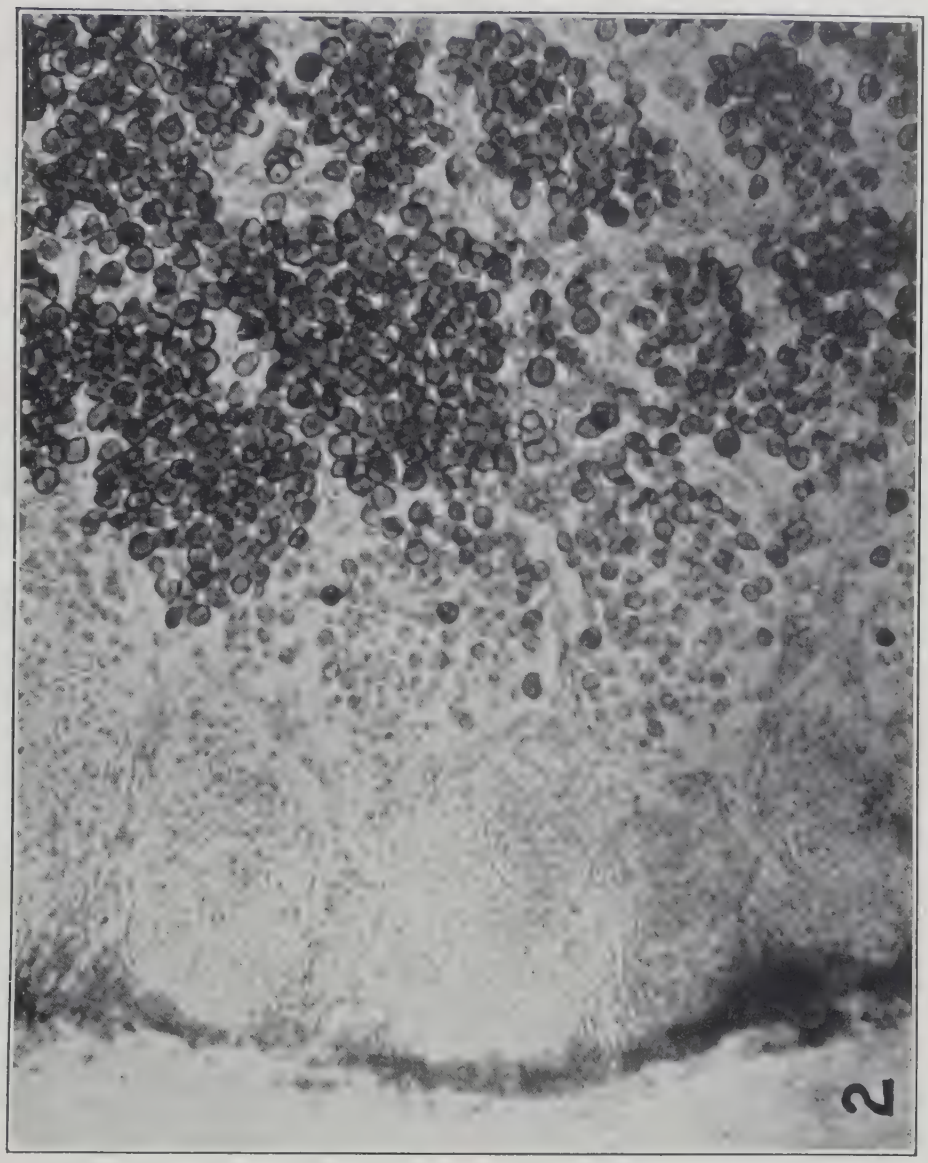
Fig. 2.—Head smut, *Sorosporium reilianum* (Kühn) McAlp., on "sumac" sorgo, San Antonio, Tex., October, 1913. Photographed by Mr. Karl F. Kellerman.

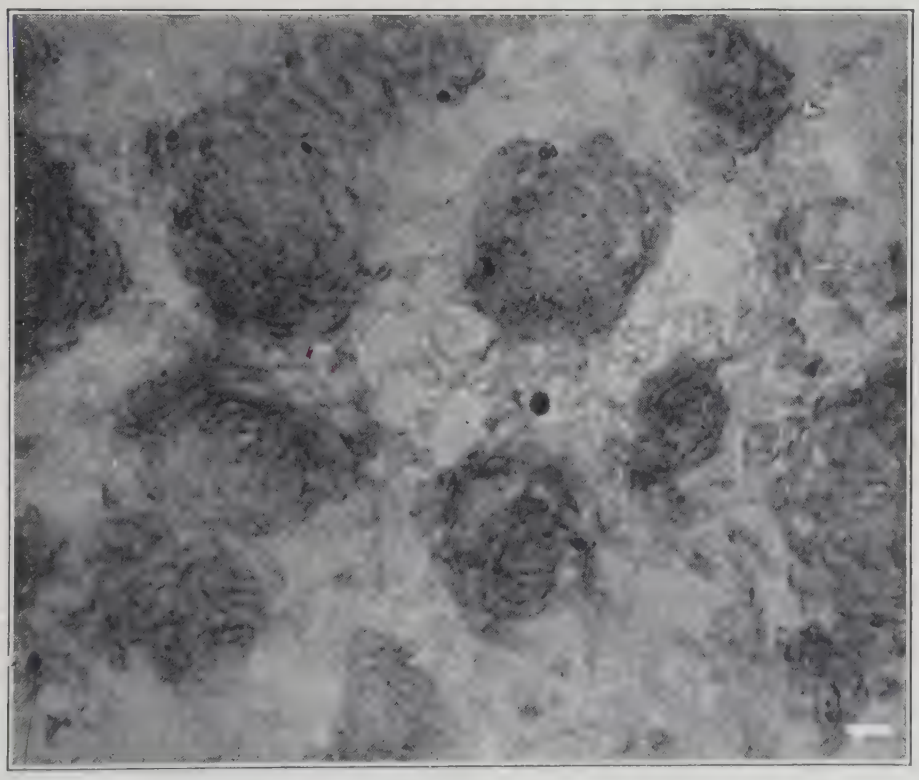
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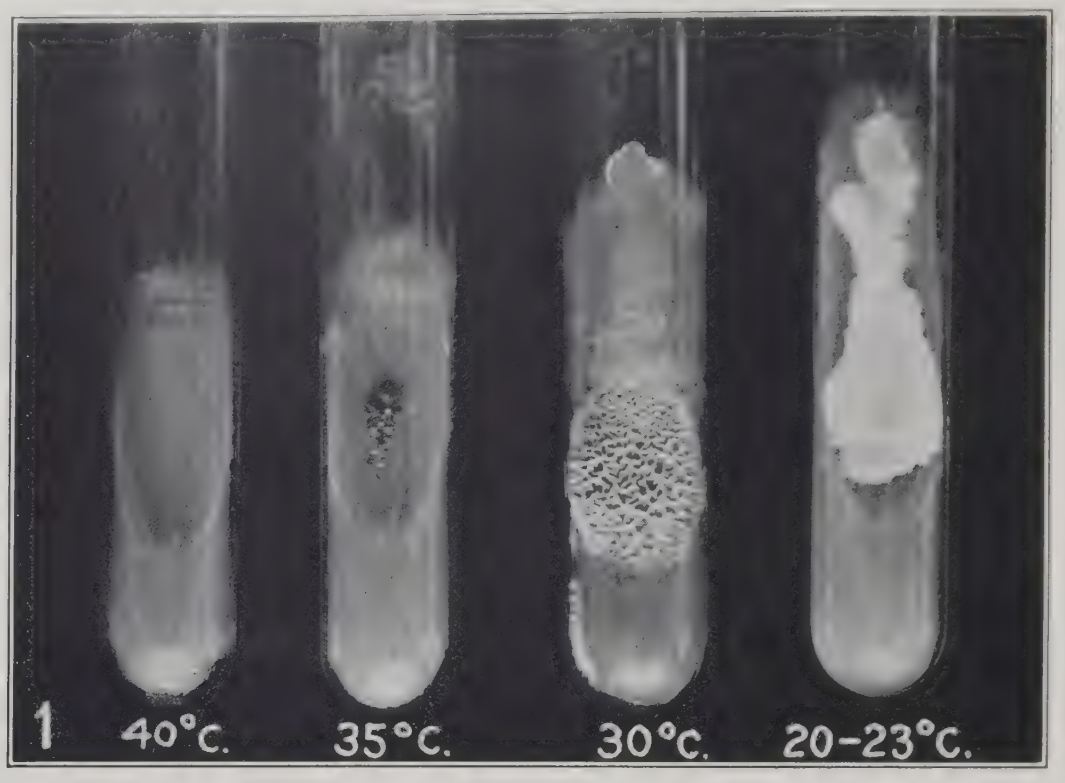
## PLATE XXXIII

Fig. 1.—Section through young sorus, showing hyphal aggregates preceding spore formation. × 710. Photomicrographed by author.

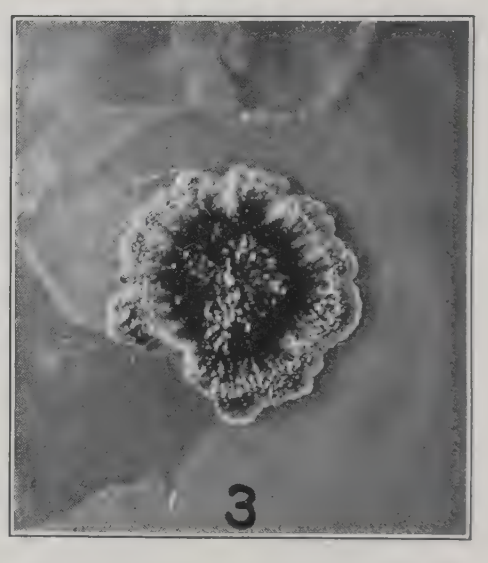
Fig. 2.—Section through immature sorus. Note the fibrovascular bundle on the left, about which the spores, none of which were as yet quite mature, were developing in groups even in the earliest stages. × 365. Photomicrographed by author.

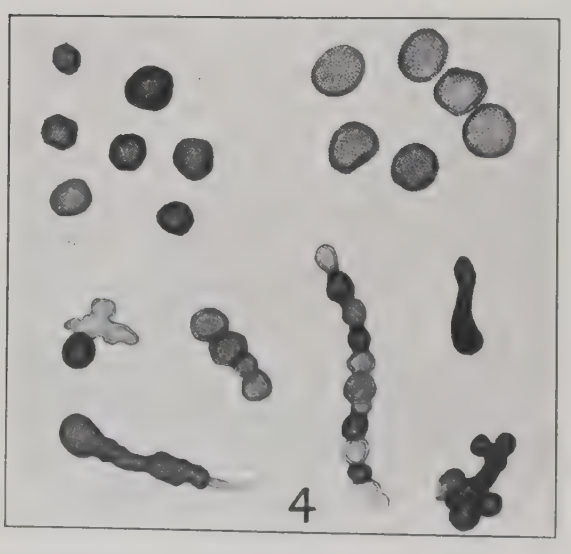

2




1
40°C.
35°C.
30°C.
20–23°C.


2


3


4

## PLATE XXXIV

Fig. 1.—Growth of organism of head-smut of sorghum and maize on carrot agar at various temperatures; cultures about 6 weeks old. × 4/5.

Figs. 2 and 3.—Twenty-two days' growth of organism of head-smut of sorghum and maize on synthetic glucose agar (fig. 2) and on carrot agar (fig. 3). Photographed by Mr. E. C. Johnson and author.

Fig. 4.—Chlamydospores of organism of head-smut of sorghum and maize formed in culture in peptonized maltose solution. In the upper right-hand corner are shown some natural spores for comparison. × 450. Drawn by author.

## PLATE XXXV

Fig. 1.—Smutted culms of Amber sorgo, showing the characteristic sterility of the main panicle. Photographed by Mr. E. C. Johnson.

Fig. 2.—Proliferated head of Blackhull kaoliang, with one normal and one smutted head. Photographed by author.

Fig. 3.—Smutted and nonsmutted plants of Red Amber sorgo used in head-smut infection experiment. Control pot (see Table V, plat B, No. 8) on left; inoculated pot (Table V, plat B, No. 3) on right, showing three smutted plants. Photographed by Mr. E. C. Johnson.


1
2
3



## PLATE XXXVI

Panicular formation in apex of proliferated sorghum flower. Longitudinal section, showing presence of hyphæ of head smut. × 70. Photomicrographed by Mr. W. W. Gilbert and author.

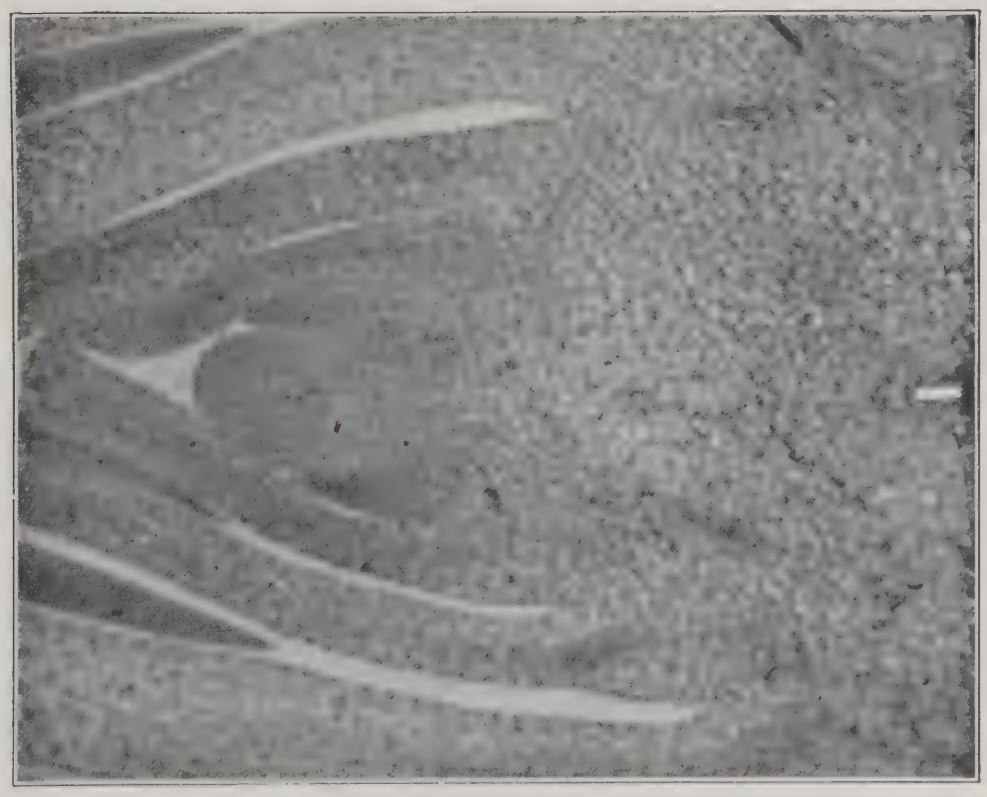
## PLATE XXXVII

Longitudinal sections through the growing points of two of the buds indicated in text figure 1, showing hyphæ of the head smut. × 150.

Fig. 1.—Bud 3 of culm B1. Positions of hyphæ are shown in text figure 2.

Fig. 2.—Bud 7 of culm N2. Photomicrographed by Mr. W. W. Gilbert and author.


2


1

# OXIDASES IN HEALTHY AND IN CURLY-DWARF POTATOES

By H. H. Bunzel,
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## INTRODUCTION

The curly-dwarf, among other related potato diseases, has been described very fully in one of the recent publications of this Department (Orton, 1914),[1] in which the very confusing literature on the subject of potato maladies referred to vaguely in the past as leaf-roll, curly-top, blight, Kräuselkrankheit, Blattrollkrankheit, etc., is critically reviewed. On the basis of this review and of the work done by the Office of Cotton and Truck Diseases and Sugar-Beet Investigations, which has made a thorough survey of the principal potato districts on this continent, as well as abroad, a number of distinct diseases are recognized, each with its characteristic symptoms and probable cause.

Some of these diseases, particularly the leaf-roll and the curly-dwarf, can not be traced to organisms of any sort for their origin and are supposedly disturbances of a purely physiological nature. To throw light on this matter, Mr. W. A. Orton, of the Bureau of Plant Industry, requested the writer to make a quantitative study of the oxidizing enzyms of potatoes at Houlton, Me., and immediate vicinity. Oxidase determinations were there carried out with healthy material, as well as with plants having the curly-dwarf disease. In this paper only such plants were considered to have curly-dwarf as showed the characteristic symptoms described by Orton (1914).

This is not the first attempt to correlate enzymatic disturbances with plant diseases. Sorauer (1908) was the first one to attribute the leaf-roll of potatoes to disturbances in the oxidase mechanism of the tubers. According to this author, the dark patches observable on the cut surfaces of such tubers are due to a greater oxidase content than is found in normal tubers; the abnormalities of the foliage are due to malnutrition through the tubers. His conclusions are based on chemical experiments of Grüss (1907). The most important and complete investigation of the subject was made by Doby (1911–12), who reached the very important conclusion that the oxidase content of the diseased tubers is greater than that of the normal ones. He also found a higher ash content and lower percentage of starch and insoluble protein in the diseased tubers, stating

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 403–404.

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Dept. of Agriculture, Washington, D. C.

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that the increased ash is probably responsible for the increased oxidase activity, judging from the work of Bertrand (1897) and others (Dony-Hénault, 1908; Dony-Hénault and Van Duuren, 1907; Trillat, 1903 and 1904). The diminution of the starch and protein in the diseased tubers is the net result of the increased combustion of their cleavage products. Doby (1911–12) measured the oxidation of pyrogallol according to the method of Bach and Chodat (1904), with and without the addition of hydrogen peroxid, and also measured the oxidation of tryosin according to an optical method (König and Krüss, 1904). In general, the peroxidase, oxygenase, and tyrosinase were present in larger quantities in the curly-dwarf tubers than in the normal ones.

With the manometric method devised by the writer, it was found that in sugar beets affected with curly-top the oxidase content of the foliage is two to three times as great as that of normal beet leaves. This same difference was found to exist when plants were studied whose growth had been retarded by causes other than the curly-top (1912).

## DESCRIPTION OF EXPERIMENTAL METHODS

For all of the experiments with leaves, fresh material was used—that is, plants collected on the day of experimentation. In the case of tubers it was sometimes necessary to allow them to lie for a few days until enough material for a complete series of experiments had accumulated. During the whole period of 10 weeks the plants were collected in the field at 7.15 a. m. and taken to the laboratory at once. The weight of the foliage was there determined and the material then ground up in a meat chopper. The juice was pressed out of the pulp by hand through a silk cloth.

Nearly all of the experiments with normal material were made on plants of one variety, the Green Mountain. With one or two intentional exceptions the samples of normal plants were taken from the same field. They were grown under fairly uniform conditions of environment, and the soil was fertilized uniformly with the same fertilizer. All of the pathological material was collected on a field several miles away, necessarily from plants of different varieties, but all grown on the same type of soil and with the same kind of fertilizer as that used in the field on which the normal plants were collected. In most cases 25-gram samples of the juice were preserved with about 100 c. c. of 25 per cent alcohol, in order that the solid contents of the juices examined might be determined, in case it seemed necessary.

The experiments were carried out in the same manner as that described in a former publication (Bunzel, 1912). The following 18 ring compounds were used as reagents to determine the oxidase activity of the juices: Benzidin, pyrogallol, alphanaphthol, leuco base of malachite green, phloroglucin, aloin, pyrocatechol, tyrosin, hydrochinone, phloridzin,

resorcin, guaiacol, orthocresol, metacresol, paracresol, orthotoluidin, metatoluidin, and paratoluidin.

In the case of each of the solid substances 0.05 gram was weighed out for each determination In the experiments with guaiacol 4 drops (0.15 gram) were used; by separate experiments it was shown that this quantity gave the highest result under the conditions of the experiments. The cresols and toluidins were found to be very poisonous, inhibiting the action of the potato oxidases when used in too great quantities. By a series of experiments it was found that 2 drops of each gave the optimum result. As in previous experiments, 1 c. c. of normal sodium hydrate was used in the glass basket in all experiments with pyrogallol, to absorb the carbon dioxid produced during the oxidation.

All of the experiments described herein were carried out at 41° C. The apparatus used were all of the small type, in which a change in pressure of 1 cm. of mercury corresponds to the absorption of 1 c. c. of oxygen. The rate of shaking was 5 complete excursions in 3 seconds. All of the results were expressed in terms of the oxidase unit previously used by the writer. The unit is an oxidase solution of such strength that 1 liter of it can bring about the oxidation of the equivalent of 1 gram of hydrogen (1912, p. 40). Blank determinations with the reagents here used showed that no measurable oxidase absorption took place under the conditions of the experiments in the absence of plant juice.

The thermostat box was provided with a false bottom about 6 inches above the floor of the box and a free space of 4 inches at each end for the sake of free circulation. The heating lamps were all arranged below this false bottom. In this way very uniform heating throughout was attained. The stopcocks were closed through an opening just large enough to admit the arm, instead of opening a window, as was done formerly. To reduce still more the disturbances of temperature within the box, the opening for the arm was protected by means of a sleeve into which the arm was slipped.

Although the results obtained with the method here used are more accurate and reliable than those obtained with any other existing method, yet this method is not entirely free from sources of error. It is probable that a part of the oxidases are destroyed by the shaking at the comparatively high temperature (41° C.). It is also probable that the reagents used for the oxidation act as poisons even in the small concentrations in which they are present. Probably, however, it will be only a matter of time before these possible sources of errors will be eliminated. For the present it may be said that the results were obtained in experiments carried out under identical conditions and are therefore comparable. In all the experiments the juice was pressed out of the ground pulp by hand and by the same operator. While it may seem that juices of more uniform composition might be obtained by pressing them out with a

machine, separate experiments show that a portion of the activity is lost thereby. The fresh hand-pressed potato juice had an activity of 0.287 units (pyrogallol), while the juice pressed out of the remaining pulp by means of a hydraulic press at a pressure up to 15 tons on a 6-inch circular ram was 0.170 units (pyrogallol) and the juice pressed out at a still higher pressure had an activity of only 0.107 units (pyrogallol). Inasmuch as the juice obtained by means of the hydraulic press had to pass through an appreciable amount of compressed pulp, it is probable that the diminished activity of the machine-pressed juice was due to the loss of a part of the oxidases by adsorption.

In this connection the results of Dixon and Atkins (1913) are very interesting. They found that in successive pressings of leaves (*Hedera helix*) in a vise, juices with increasing concentration of electrolytes were obtained. They experimented also with leaves treated with liquid air and concluded that the only way to obtain juices corresponding to the concentration of the sap in the vacuoles of the uninjured tissues is to press them out after exposure to liquid air. Unfortunately, such procedure was impossible during this work, which had to be carried out in the field.

## RATE OF GROWTH OF THE POTATO PLANT

In former publications it has been shown that the oxidase content of juices bears a very definite relation to the rate of development of the particular plant specimens from which they are derived. In the sugar beet, which the writer studied in this respect, the oxidase content of the foliage runs up appreciably when the normal growth of the plants is interfered with by drought, excessive watering, diseases, etc. In the foliage of normally developing sugar-beet plants the oxidase content of the juice is only about one-half that of stunted plants. On the basis of the results obtained with sugar beets the following generalizations can be made:

Normal growth.....Normal (low) oxidase content.
Retarded growth.....Abnormal (high) oxidase content.

The recognition of this fact led to an examination of the rate of growth of the potato plants which were used in this research. Table I shows the relation which the size of the shoots and the foliage of all the plants in a hill, as well as of the single shoots, bears to the age of the plants.

TABLE I.—*Relation of the total weight of the shoots of the whole hills, as well as of the single shoots, to the age of the potato plants*

| Series No. | Date of collection. | Age. | Total weight of shoots. | Number of hills. | Mean total weight of shoots per hill. | Number of shoots. | Mean weight of shoots. |
|---|---|---|---|---|---|---|---|
| | | *Days.* | *Grams.* | | *Grams.* | | *Grams.* |
| 1 | July 9 | 29 | 84 | 5 | 17 | ..... | ..... |
| 2 | July 10 | 30 | 102 | 7 | 15 | 17 | 6 |
| 4 | July 11 | 31 | 150 | 8 | 19 | 22 | 6.8 |
| 6 | July 12 | 32 | 108 | 8 | 13.5 | 19 | 5.7 |
| 9 | July 14 | 34 | 222 | 15 | 15 | 32 | 6.9 |
| 10 | July 15 | 35 | 297 | 9 | 33 | 23 | 12.9 |
| 12 | July 31 | 19 | 75 | 7 | 11 | 40 | 1.9 |
| 14 | July 31 | 19 | 100 | 6 | 17 | 41 | 2.4 |
| 15 | Aug. 1 | 20 | 170 | 6 | 28 | 64 | 2.7 |
| 18 | Aug. 2 | 60 | 360 | 1 | 360 | 3 | 120 |
| 21 | Aug. 4 | 62 | 750 | 2 | 375 | 4 | 188 |
| 24 | Aug. 8 | 66 | 680 | 1 | 680 | 4 | 170 |
| 26 | Aug. 9 | 67 | 368 | 1 | 368 | 1 | 368 |
| 29 | Aug. 11 | 30 | 365 | 5 | 73 | 44 | 8.3 |
| 32 | Aug. 21 | 40 | 500 | 1 | 500 | 7 | 71.4 |
| 35 | Aug. 29 | 88 | 350 | 1 | 350 | 1 | 350 |
| 38 | Sept. 8 | 98 | 375 | 1 | 375 | 1 | 375 |

In order to present these data more clearly, they were plotted as shown in figure 1. The ages of the plants are measured off on the abscissæ and the weight of the shoots on the ordinates. The continuous line corresponds to the development of the plants (the total weight of the shoots of one hill), and the broken line corresponds to the mean rate of development of all of the single shoots of one hill.

The irregularities of the curve representing the growth of the shoots of a whole hill are apparently due to variations in the number of stalks contained therein. This becomes strikingly apparent from the smoothness of the curve representing the growth of single stalks. With practically no interruption this curve shows a gradual increase in size until the sixty-seventh day is reached, growth of the stalks apparently stopping at that point. The curve from this point on is practically a straight line.

## OXIDASES OF HEALTHY POTATO PLANTS

In order to be able to compare the oxidase activities of diseased potato plants with healthy ones at the same stage of development, it was essential to establish the oxidase content of healthy material at all stages of development. Such a study on normal plants is also of general physiological interest. While the excellent work of Palladin (1906) and his school has shown that the respiration of plants takes place in stages corresponding to several distinct respiratory enzyms, they have made no measurements of the oxidizing power of these respiratory enzyms. Moreover, working with frozen wheat seedlings and those not frozen and with etiolated leaves of *Vicia faba* and leaves of *Plectogyne japonica*, they con-

clude that "oxidase" and "oxygenase" are practically lacking in embryonic organs and that their concentration in these plants rises during growth and diminishes again when growth has stopped. They draw their conclusions entirely from the quantity of $CO_2$ liberated by the

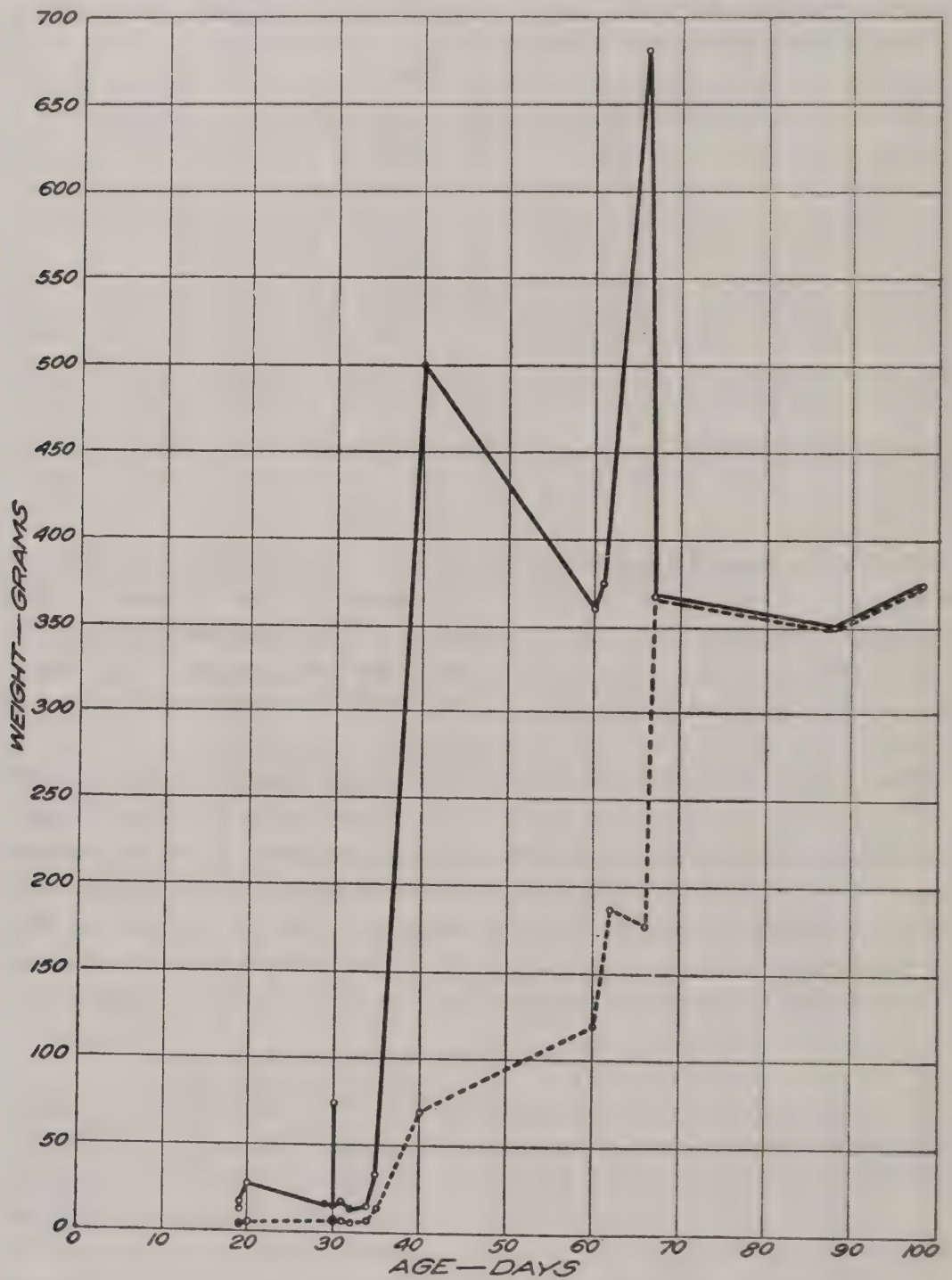



FIG. 1.—Rate of development of the aerial portion of potato plants and mean rate of development of all the single shoots in one hill.

plant organs under different conditions. It seemed of great interest, therefore, to find out what relation the concentrations of the oxidases present in the pressed-out sap of a plant bear to the state of development of the same plant.

## OXIDASE ACTIVITY OF THE JUICE OF THE SHOOTS

The results obtained in the measurement of the oxidases in the juice of healthy potato plants of the same variety at various ages, grown under normal and as nearly identical conditions as possible, are given in Tables II to VIII, and some of the results are also shown graphically in figures 2 to 20.

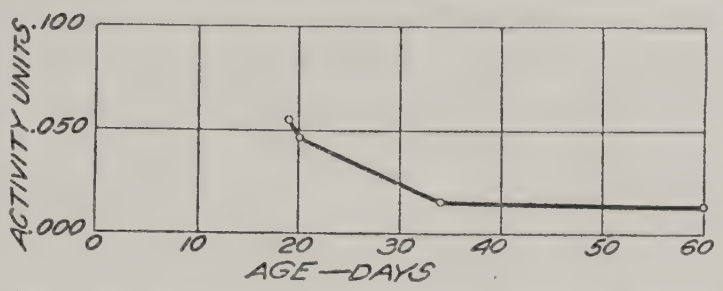



FIG. 2.—Curve showing oxidation of leuco base of malachite green in the presence of the juice of green potato shoots.

The shoots were taken from the plants immediately above the point where they emerged from the soil. In figures 2 to 5 [1] the abscissæ represent the age of the plants as measured from the time of planting, and the ordinates the activities of the juices as measured in the oxidation of the various aromatic compounds used. These data show a distinct downward tendency; there is apparently a marked diminution in the oxidase activities of the pressed-out juice of the shoots during the beginning of their growth.

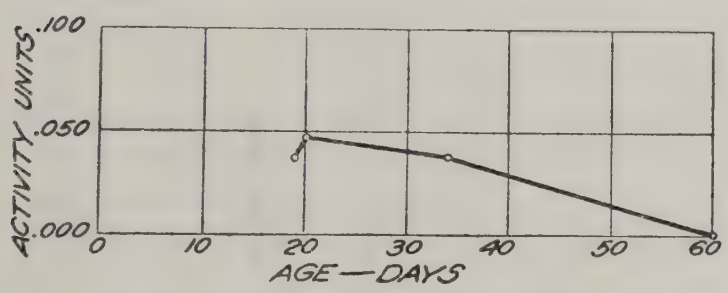



FIG. 3.—Curve showing oxidation of aloin in the presence of the juice of green potato shoots.

## OXIDASE ACTIVITY OF THE JUICE OF THE STEMS

Experiments on sugar beets showed that the juice obtained from the stems of the plants exhibited very much less oxidase activity than that of the leaves (Bunzel, 1913a; 1913b). It seemed probable, therefore, that the juice of the stems of the potato plants examined would also be less active than the juice of the leaves. A comparative increase

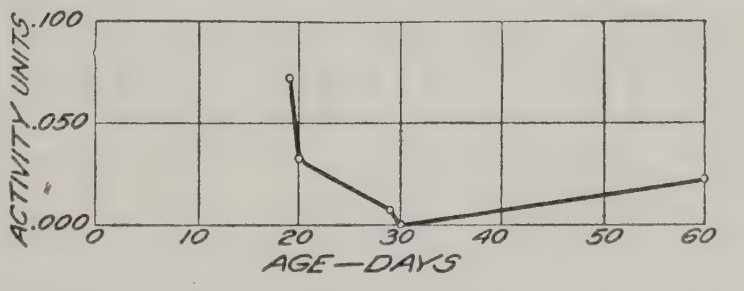



FIG. 4.—Curve showing oxidation of tyrosin in the presence of the juice of green potato shoots.

[1] Inasmuch as nearly all of the curves obtained in working with the 18 reagents show the same general relationships, for the sake of a briefer and, therefore, more comprehensive presentation of the facts, only 4 to 8 curves are presented in the case of the shoots, foliage, and tubers, respectively. While these curves were not picked at random, they are typical of the situation in each case. The writer felt justified in doing this, inasmuch as all of the curves can be constructed from the tables in the text, and since the results are numerically compared in Table XII.

TABLE II.—*Oxidase activities of the juice of shoots of healthy potato plants*

| Series No. | Date. | Number of hills used. | Total weight of shoots. | Mean total weight of shoots per hill. | Date of planting. | Age of plant. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | O-cresol. | M-cresol. | P-cresol. | O-toluidin. | M-toluidin. | P-toluidin. |
| | | | *Grams.* | *Grams.* | | *Days.* | | | | | | | | | | | | | | | | | | |
| 1 | July 9 | 5 | 84 | 17 | June 10 | 29 | | | | | | | | 0.008 | 0.078 | | | | | | | | | |
| 2 | July 10 | 7 | 102 | 15 | June 10 | 30 | | | | | | | 0.047 | 0 | .320 | | | | | | | | | |
| 3 | | | | | | | 0 | | | | | | | | | | | | | | | | | |
| 4 | July 11 | 8 | 150 | 19 | June 10 | 31 | | | 0 | | 0.008 | | | | .296 | | | 0.117 | | | | | | |
| 5 | | | | | | | | | | | | | | | | 0.179 | | | | | | | | |
| 6 | | | | | | | | | | | | | | | | | | | | 0.281 | | | | |
| 7 | July 12 | 8 | 108 | 13.5 | June 10 | 32 | | | | | | | | | | | | | 0.039 | | | | | |
| 8 | | | | | | | | | | | | | | | | | | | | | 0.523 | | | |
| 9 | July 14 | 15 | 222 | 15 | June 10 | 34 | | | | 0.016 | | 0.039 | | | | | | | .105 | .382 | .507 | | | |
| 10 | July 15 | 9 | 297 | 33 | June 10 | 35 | | | | | | | | | | | 0 | | | | | 0.062 | 0.047 | 0.039 |
| 11 | | | | | | | | | | | | | .031 | | .476 | | | | | | | | | |
| 12 | July 31 | 7 | 75 | 11 | July 12 | 19 | | | | | | | .023 | .074 | .553 | .293 | .078 | .129 | | | | | | |
| 13 | | | | | | | .055 | 0 | .047 | .055 | .047 | .039 | | | | | | | | | | | | |
| 14 | July 31 | 6 | 100 | 17 | July 12 | 19 | | | | | | | | | | | | | .086 | .312 | .491 | | | |
| 15 | | | | | | | .074 | .016 | .016 | .047 | .047 | .047 | | | | | | | | | | | | |
| 16 | Aug. 1 | 6 | 170 | 28 | July 12 | 20 | | | | | | | .062 | .031 | .534 | .215 | .047 | .086 | | | | | | |
| 17 | | | | | | | | | | | | | | | | | | | .047 | .218 | .406 | 0 | .047 | .023 |
| 18 | | | | | | | | | | | | | | | | | | | | | | | | |
| 19 | Aug. 2 | 1 | 360 | 360 | June 3 | 60 | .016 | 0 | .016 | .012 | .020 | 0 | .023 | .023 | .226 | .086 | .031 | .020 | | | | | | |
| 20 | | | | | | | | | | | | | | | | | | | .043 | .078 | .152 | .023 | .039 | .023 |

[a] Leuco base of malachite green.

in size of the stem of a growing plant as compared with the foliage or a diminution with age of the activity of the stem juice as compared with the activity of the foliage juice would therefore result in a diminution with age of the activity of the juice of the aboveground portion of the plant. Consequently oxidase determinations were made on the juice obtained from stems of plants 69 days old. The results are given in Table III.

TABLE III.—*Oxidase activities of the juice of stems of plants 69 days old*

| Reagent. | Activity.[a] | Reagent. | Activity.[a] |
|---|---|---|---|
| Benzidin | 0.035 | Phloridzin | 0.078 |
| Pyrogallol | 0 | Resorcin | 0 |
| α-naphthol | 0 | Guaiacol | .023 |
| Leuco base of malachite green | 0 | O-cresol | 0 |
| Aloin | .031 | M-cresol | .101 |
| Phloroglucin | 0 | P-cresol | .168 |
| Pyrocatechol | .023 | O-toluidin | 0 |
| Tyrosin | 0 | M-toluidin | 0 |
| Hydrochinone | 0 | P-toluidin | 0 |

[a] Activity expressed in units as measured in the oxidation of the reagents.

The stem juice proved to have no activity whatever toward 11 of these 18 reagents, and toward the remaining 7 it was slight in comparison with the activity of the foliage juice, as will be shown later. That the stem during growth increased in weight more rapidly than the remainder of the shoot is shown in Table IV (column 9). It is probable, therefore, that the diminishing activity of the juice of the shoots of the potato plant was due to increasing dilution with age of the very active leaf juice with the relatively inactive stem juice.

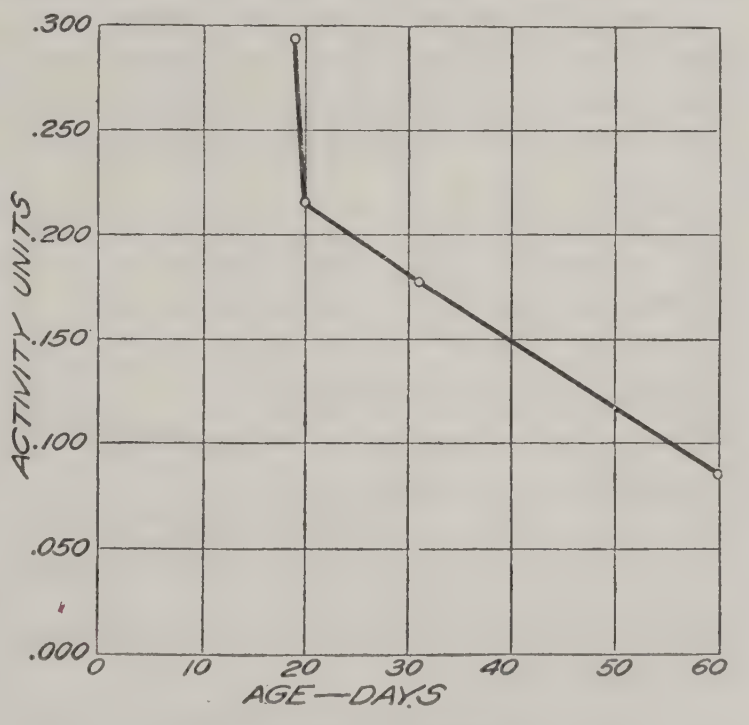



FIG. 5.—Curve showing oxidation of phloridzin in the presence of the juice of green potato shoots.

## OXIDASE ACTIVITY OF THE JUICE OF THE LEAVES

The leaves proper are the seat of the greatest physiological activity in plants. The food of the plant is largely synthesized in the leaves and also in part broken down in them, according to the needs of the plant.

In physiological disturbances, such as the curly-dwarf disease of potatoes and the curly-top of sugar beets appear to be, the leaves are the parts primarily affected. It is therefore to be expected that any chemical differences existing between healthy plants and plants affected with the curly-dwarf disease will be most pronounced in the leaves proper. In order to be certain of results which represent the activity of the juice of

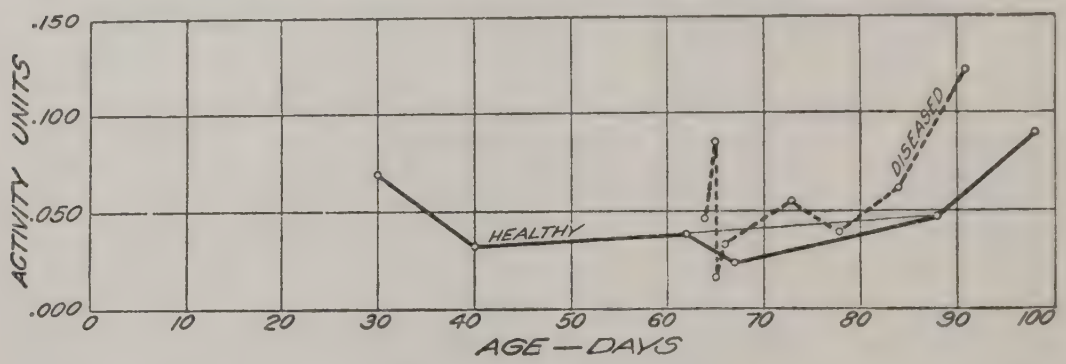



FIG. 6.—Curve showing oxidation of leuco base of malachite green in the presence of the juice of potato foliage.

the foliage proper, all experiments with the green parts of the potato plant were from this point on carried out on leaves alone. Table IV gives the data on the activity of the leaf juice alone.

Some of these results are also graphically presented in figures 6 to 12. (See footnote, p. 379.) For easy comparison the figures obtained with healthy leaves, as well as those obtained later with curly-dwarf leaves, were plotted on the same systems of coordinates. The continuous lines

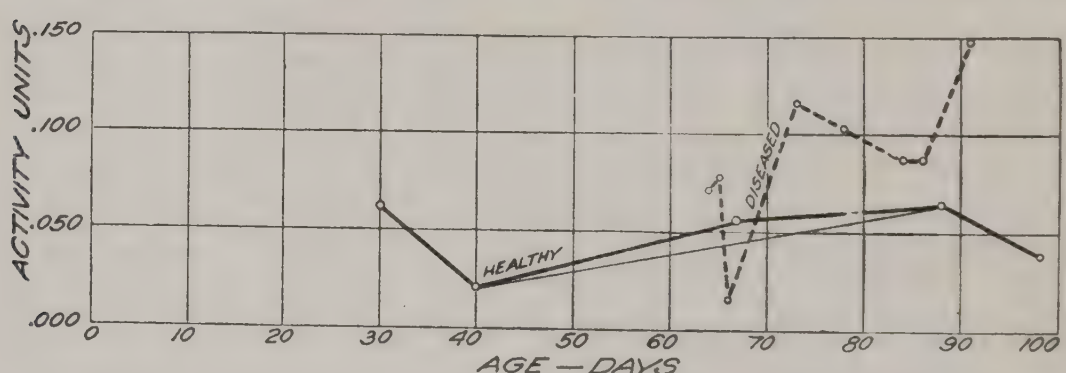



FIG. 7.—Curve showing oxidation of pyrocatechol in the presence of the juice of potato foliage.

represent the results obtained with healthy plants, the dotted lines those with the diseased ones.

These curves show great fluctuations of oxidase content. Barring some of the irregularities, probably due to individual peculiarities of the samples examined, the curves take a downward direction at first, remain at a low level for a prolonged period, and take an upward movement again towards the end. The lowest point in the curve is reached generally on the fortieth day; the period of low oxidase content extends to a point of time between the sixtieth and eightieth day, when the upward movement of

TABLE IV.—*Oxidase activities of the juice of the leaves of healthy potato plants*

| Series No. | Date. | Number of hills used. | Total weight of shoots. | Mean total weight of shoots per hill. | Date of planting. | Age of plant. | Total weight of stems. | Stems × 100. Total weight of shoots. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | O-cresol. | M-cresol. | P-cresol. | O-toluidin. | M-toluidin. | P-toluidin. |
| | | | *Grams.* | *Grams.* | | *Days.* | *Grams.* | *Grams.* | | | | | | | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | | | | | 0.043 | 0.456 | 0.109 | 0.059 | 0.059 | | | | | | |
| 22 | Aug. 4 | 2 | 750 | 375 | June 3 | 62 | 402 | 54 | 0.055 | 0.016 | 0.035 | 0.039 | 0.035 | 0.047 | | | | | | | 0.004 | 0.191 | 0.308 | 0.020 | 0.020 | 0.035 |
| 23 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 24 | Aug. 8 | 1 | 680 | 680 | June 3 | 66 | 400 | 59 | | | | | | | | | .304 | .086 | .031 | .062 | .031 | | | .039 | .031 | .039 |
| 25 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 26 | | | | | | | | | | | | | | | 0.055 | .062 | .601 | .172 | .094 | .094 | | | | | | |
| 27 | Aug. 9 | 1 | 368 | 368 | June 3 | 67 | 204 | 55 | | | | | | | | | | | | | .059 | .086 | .211 | .078 | .078 | .062 |
| 28 | | | | | | | | | .062 | | 0 | .023 | .055 | .078 | | | | | | | | | | | | |
| 29 | | | | | | | | | .105 | .016 | .035 | .070 | .070 | .094 | | | | | | | | | | | | |
| 30 | Aug. 11 | 5 | 365 | 73 | July 12 | 30 | 170 | 47 | | | | | | | .062 | .094 | | .250 | .094 | .191 | | | | | | |
| 31 | | | | | | | | | | | | | | | | | | | | | .082 | .257 | .398 | | .078 | .098 |
| 32 | | | | | | | | | .039 | .023 | 0 | .031 | .016 | .055 | | | | | | | | | | | | |
| 33 | Aug. 21 | 1 | 500 | 500 | July 12 | 40 | 245 | 49 | | | | | | | .020 | .035 | .222 | .078 | .016 | .039 | | | | | | |
| 34 | | | | | | | | | | | | | | | | | | | | | .020 | .047 | .117 | 0 | .031 | .055 |
| 35 | | | | | | | | | .059 | | 0 | .047 | .031 | .051 | | | | | | | | | | | | |
| 36 | Aug. 29 | 1 | 350 | 350 | June 2 | 88 | 180 | 51 | | | | | | | .062 | | .296 | .070 | .055 | .094 | | | | | | |
| 37 | | | | | | | | | | | | | | | | | | | | | .051 | .098 | .152 | .070 | .078 | .062 |
| 38 | | | | | | | | | .094 | .039 | .039 | .090 | .086 | .121 | | | | | | | | | | | | |
| 39 | Sept. 8 | 1 | 375 | 375 | June 2 | 98 | 235 | 63 | | | | | | | .039 | .101 | .425 | .187 | .094 | .062 | | | | | | |
| 40 | | | | | | | | | | | | | | | | | | | | | .082 | .094 | .242 | .062 | .101 | .062 |

[a] Leuco base of malachite green.

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the curve generally begins. The juice from the plant collected on the sixty-seventh day seems unusually rich in oxidases. If the points obtained from the data on this plant were discarded, the curves would be all quite regular, with the exception of those corresponding to the oxidation of hydrochinone and of some of the cresols. To show what types of

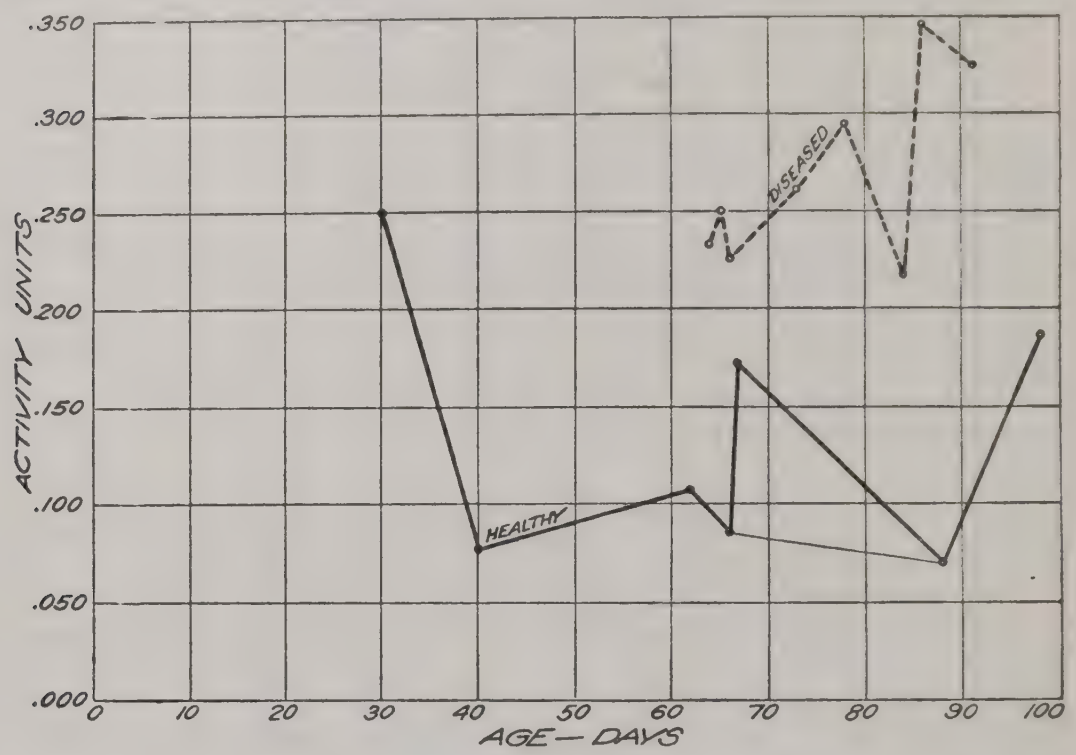



FIG. 8.—Curve showing oxidation of phloridzin in the presence of the juice of potato foliage.

curves would be obtained by elimination of the points obtained for the sixty-seventh day of growth, which point seems irregular, the adjacent points on both sides of the "sixty-seventh-day point" are connected with relatively thin lines to complete the curves; the initial fall and the final rise thus become very apparent and clear-cut.

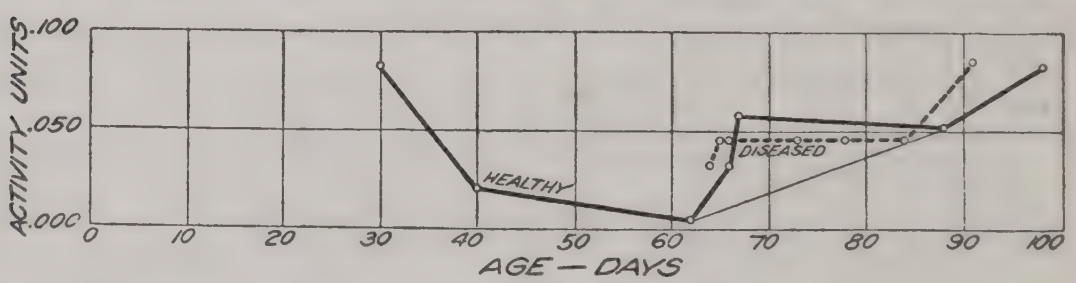



FIG. 9.—Curve showing oxidation of o-cresol in the presence of the juice of potato foliage.

The curves, of course, are not smooth. This is to be expected when it is considered that there are numerous factors influencing the physiological condition of the plants. Differences in the nature of the seed, of the soil, and many other factors probably influence the development of the plant qualitatively as well as quantitatively.

With all of the reagents except guaiacol and metacresol, the rise in the oxidase content observed during the second half of the period of examination coincides approximately with the stoppage of growth, which point is shown in figure 1 to be about the sixty-seventh day. In this respect, therefore, these results are in striking harmony with those obtained while working on diseased sugar beets. In the case of sugar beets the writer has shown that the factors which had a retarding influence on the growth of the plants also caused the oxidase content of the juice of their foliage

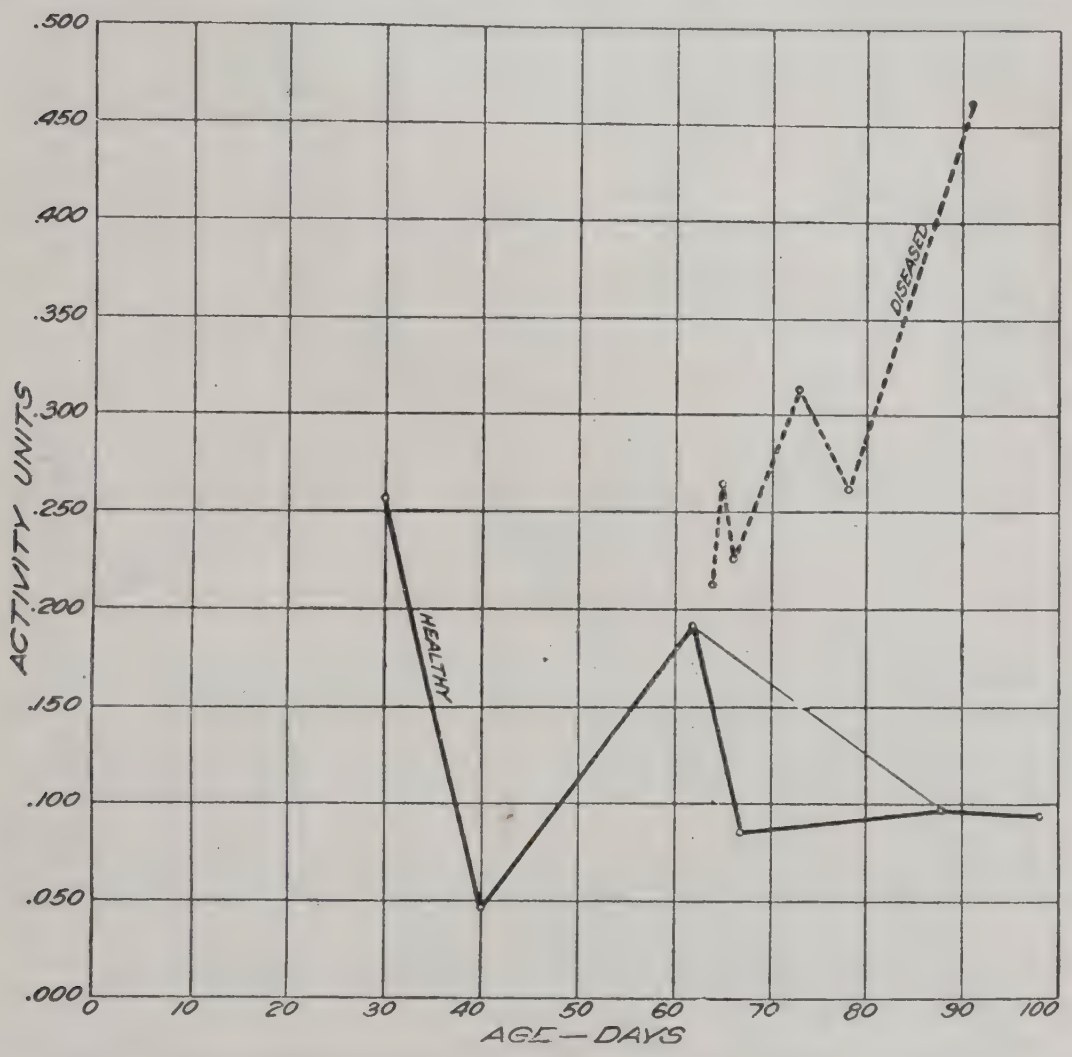



FIG. 10.—Curve showing oxidation of m-cresol in the presence of the juice of potato foliage.

to increase. It is possible that the same factors which led to an increased oxidase content during the retardation of growth of sugar beets will lead to a similar rise during normal cessation of growth in potato plants.

## OXIDASE ACTIVITY OF THE JUICE OF THE SPROUTS AND OF THE TUBERS FROM WHICH THE SPROUTS HAD BEEN REMOVED

The relatively high oxidase content of very young potato plants suggested an examination of the sprouts from the tubers. Seed tubers of the Green Mountain variety of the same stock as was used for all of the

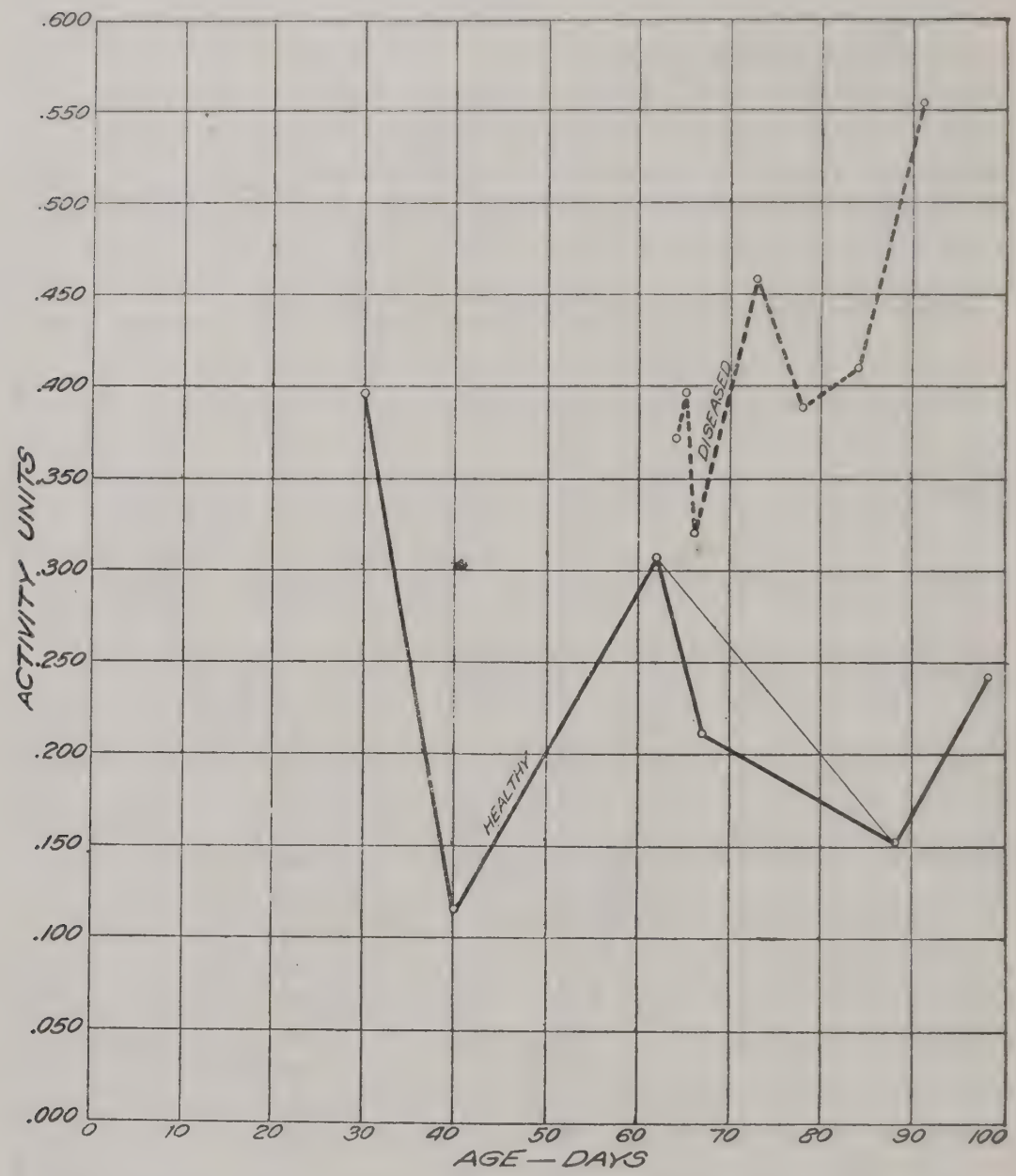



FIG. 11.—Curve showing oxidation of p-cresol in the presence of the juice of potato foliage.

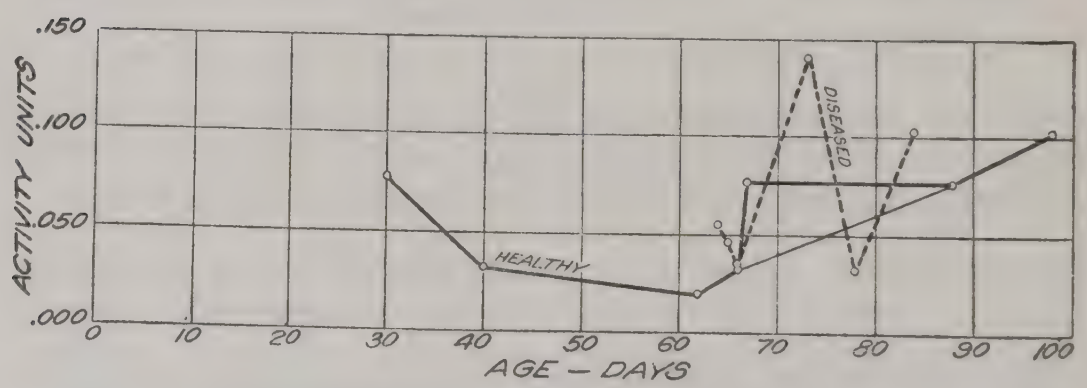



FIG. 12.—Curve showing oxidation of m-toluidin in the presence of the juice of potato foliage.

experiments already described in this paper were stored at room temperature from July 12 to September 3, 1913. During that time the tubers were lying on a table exposed to the light, and the temperature during the period fluctuated between 15° and 25° C. There were 36 tubers, soft but sound, yielding 170 grams of sprouts; the 36 tubers from which the sprouts had been removed weighed 2,670 grams. Only 22 c. c. of juice were obtained from the sprouts in the usual way. This juice turned immediately to a chocolate-brown color. The oxidase activity of the juice of the sprouts toward seven of the reagents is given in Table V. The oxidase activity of the juice of the tubers toward all of the reagents is given in Table VI.

TABLE V.—*Oxidase activities of the juice of the sprouts from Green Mountain seed-potato tubers*

| Series No. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pyrocatechol. | Tyrosin. | Phloridzin. | Resorcin. | Hydrochinone. | Guaiacol. | Paracresol. |
| 41 | 0. 562 | 0. 296 | 0. 359 | 0. 055 | ..... | ..... | ..... |
| 42 | ..... | ..... | ..... | ..... | 0. 807 | 0. 488 | 0. 495 |

[1] Leuco base of malachite green.

## OXIDASE ACTIVITY OF THE JUICE OF THE TUBERS

The method of procedure in the case of the potato tubers was the same as that used with the foliage. The tubers were freed from adhering soil by means of cold, running water and were wiped dry with a clean towel and ground up whole. It has been known for some time that the juice obtained from the layers of the potato tuber near the surface is more active than that from the inner portions. Notwithstanding this variation of oxidase activity in different parts of the tuber, the whole tubers, including the peel, were used for these experiments. This was done to avoid the introduction of new factors. The results are presented in Table VII.

To see whether the oxidase content of the juice from the tubers bears any relation to either the age of the plant from which the tubers are derived or their own weight, the ratio of oxidase content to age and weight was represented graphically. In the figures 13 to 20 (see footnote, p. 379) two sets of curves are given. In both sets the oxidase activities of the juices are shown on the ordinates, while the ages and weights, respectively, are laid off on the abscissæ. The curves formed by the continuous lines show the relation of age to oxidase content and the curves formed by the thin broken lines show the relation of the size of the tubers to their oxidase content.

From these curves no definite relationship is apparent between the oxidase content of the tubers on the one hand and their age or size on

TABLE VI.—*Oxidase activities of the juice of healthy potato tubers which sprouted in the laboratory and from which the sprouts had been removed*

| Series No. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | Orthocresol. | Metacresol. | Paracresol. | Orthotoluidin. | Metatoluidin. | Paratoluidin. |
| 43 | 0.332 | 0.203 | 0.140 | 0.012 | 0.047 | 0.113 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 44 | ..... | ..... | ..... | ..... | ..... | ..... | 0.413 | 0.250 | 0.710 | 0.273 | 0.023 | 0.382 | ..... | ..... | ..... | ..... | ..... | ..... |
| 45 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0 | 0.257 | 0.952 | 0 | 0.012 | 0.156 |

[a] Leuco base of malachite green.

TABLE VII.—*Oxidase activities of the juice of healthy potato tubers*

| Series No. | Series No. of leaves of same plant. | Date of collection. | Date of experiment. | Number of hills used. | Total number of shoots. | Total number of tubers. | Number of tubers per hill. | Number of tubers per shoot. | Total weight of tubers. | Average weight of tubers. | Age of plant. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | O-cresol. | M-cresol. | P-cresol. | O-toluidin. | M-toluidin. | P-toluidin. |
| | | | | | | | | | *Grams.* | *Grams.* | *Days.* | | | | | | | | | | | | | | | | | | |
| 46 | 21 | Aug. 4 | Aug. 13 | 2 | 4 | 13 | 6.5 | 3.2 | 114 | 8.8 | 62 | 0.121 | 0.137 | 0.070 | ..... | 0 | 0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 47 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | 0.281 | 0.082 | ..... | 0.257 | 0 | 0.218 | ..... | ..... | ..... | ..... | ..... | ..... |
| 48 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 49 | 32 | Aug. 21 | Aug. 22 | 5 | 32 | 101 | 20.2 | 3.2 | 565 | 5.6 | 40 | .265 | .218 | .117 | (?)0.016 | .047 | .023 | ..... | ..... | ..... | ..... | ..... | ..... | 0 | 0.371 | 0.515 | 0 | 0 | 0.043 |
| 50 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | .367 | .140 | 0.367 | .281 | .047 | .312 | ..... | ..... | ..... | ..... | ..... | ..... |
| 51 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .055 | ..... | .507 | 0 | .023 | .094 |
| 52 | | | | | | | | | | | | ..... | .203 | .031 | .023 | .027 | .020 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 53 | 35 | Aug. 29 | Aug. 30 | 1 | 1 | 5 | 5 | 5 | 470 | 94.0 | 88 | ..... | ..... | ..... | ..... | ..... | ..... | .172 | 0 | .476 | .211 | .008 | .062 | ..... | ..... | ..... | ..... | ..... | ..... |
| 54 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .016 | ..... | .655 | 0 | .008 | .008 |
| 55 | | | | | | | | | | | | .179 | .191 | .172 | .008 | .016 | .047 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 56 | 38 | Sept. 8 | Sept. 9 | 1 | 1 | 4 | 4 | 4 | 240 | 60.0 | 98 | ..... | ..... | ..... | ..... | ..... | ..... | .226 | .086 | .386 | .242 | .008 | .211 | ..... | ..... | ..... | ..... | ..... | ..... |
| 57 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0 | .406 | .831 | 0 | 0 | 0 |

[a] Leuco base of malachite green.

the other. The irregularities are due, no doubt, to differences between individual samples and to slight differences in the mode of preparation of the juice. Great care was used to maintain uniformity of technique throughout this work, so that it does not seem very likely that the latter factor plays a rôle in the variations of oxidase contents observed.

## OXIDASE ACTIVITY OF THE JUICE OF SEED TUBERS

In connection with these results the oxidase activity of the seed tubers of the plants used in these experiments seemed of interest. The average weight of the tubers when examined was 90 grams. The oxidase determinations were started on July 18, when the tubers had just begun to sprout. The results are given in Table VIII.

TABLE VIII.—*Oxidase activities of the juice of seed potato tubers from which all the healthy material used in this investigation was obtained*

| Series No. | Date. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. |
| 58 | July 18 | ..... | ..... | ..... | ..... | ..... | ..... | 0.261 | 0.109 | 0.546 |
| 59 | July 18 | 0.179 | ..... | ..... | ..... | ..... | 0.031 | ..... | ..... | ..... |
| 60 | July 19 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 61 | July 19 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 62 | July 19 | .226 | ..... | ..... | ..... | ..... | .055 | ..... | ..... | ..... |
| 63 | July 24 | ..... | ..... | ..... | ..... | 0.027 | ..... | ..... | ..... | ..... |
| 64 | July 24 | ..... | 0.254 | 0.125 | 0.086 | ..... | ..... | ..... | ..... | ..... |

| Series No. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Phloridzin. | Resorcin | Guaiacol. | Orthocresol. | Metacresol. | Paracresol. | Orthotoluidin. | Metatoluidin. | Paratoluidin. |
| 58 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 59 | ..... | ..... | 0.312 | ..... | ..... | ..... | ..... | ..... | ..... |
| 60 | ..... | ..... | ..... | ..... | ..... | ..... | 0.008 | 0.000 | 0.094 |
| 61 | ..... | ..... | ..... | 0.047 | 0.530 | 0.913 | ..... | ..... | ..... |
| 62 | ..... | ..... | .257 | ..... | ..... | ..... | ..... | ..... | ..... |
| 63 | 0.359 | 0.008 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 64 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

[a] Leuco base of malachite green.

## OXIDASES OF CURLY-DWARF-DISEASED POTATO PLANTS

The plot of potatoes from which all the normal material was collected turned out to be remarkably free from curly dwarf. The pathological material was therefore collected on a larger field several miles remote from the other. About 10,000 different kinds of potatoes were grown in the field, and on account of the comparative scarcity of the pathological material samples were chosen from a number of different varieties. In all of the experiments the name of the variety is stated whenever it is known. The conditions of growth with reference to soil and atmospheric conditions were practically the same in the case of both the diseased and healthy potatoes.

TABLE IX.—*Oxidase activities of the juice of shoots of curly-dwarf potato plants*

| Series No. | Date. | Number of variety. | Name of variety. | Number of plants. | Total weight of shoots. | Mean total weight of shoots per hill. | Date of planting. | Age of plant. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | O-cresol. | M-cresol. | P-cresol. | O-toluidin. | M-toluidin. | P-toluidin. |
| | | | | | *Gms.* | *Gms.* | | *Days.* | | | | | | | | | | | | | | | | | | |
| 65 | July 17 | Unknown. | Unknown | 10 | 361 | 36 | June 2 | 45 | ..... | 0.043 | ..... | ..... | ..... | ..... | 0.078 | 0.070 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 66 | | | | | | | | | ..... | .031 | ..... | ..... | ..... | 0.039 | .082 | .066 | 0.359 | ..... | ..... | 0.125 | ..... | ..... | ..... | ..... | ..... | ..... |
| 67 | July 21 | ...do | ...do | 10 | 435 | 44 | ...do... | 49 | ..... | ..... | ..... | ..... | ..... | .062 | ..... | ..... | .406 | ..... | ..... | .094 | ..... | ..... | ..... | ..... | ..... | ..... |
| 68 | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.023 | 0.312 | 0.445 | ..... | ..... | ..... |
| 69 | July 22 | ...do | ...do | 7 | 260 | 37 | ...do... | 50 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.047 | 0.057 | 0.117 |
| 70 | July 23 | ...do | ...do | 7 | 260 | 37 | ...do... | 51 | ..... | ..... | ..... | ..... | 0.031 | ..... | ..... | ..... | ..... | 0.226 | 0.047 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 71 | | | | | | | | | 0.074 | ..... | 0.023 | 0.047 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 72 | July 28 | ...do | ...do | 3 | 111 | 37 | ...do... | 56 | .062 | ..... | ..... | ..... | .020 | ..... | .031 | .047 | ..... | ..... | .043 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 73 | | | | | | | | | ..... | ..... | 0 | .016 | ..... | .020 | ..... | ..... | .374 | .187 | ..... | .078 | ..... | ..... | ..... | ..... | ..... | ..... |
| 74 | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .039 | .140 | .382 | 0 | 0 | .012 |
| 75 | July 29 | ...do | ...do | 4 | 370 | 93 | ...do... | 57 | ..... | ..... | ..... | ..... | ..... | ..... | .078 | .023 | .452 | .371 | .031 | .078 | ..... | ..... | ..... | ..... | ..... | ..... |
| 76 | | | | | | | | | .062 | ..... | .055 | .016 | .023 | .023 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 77 | July 30 | 6652 | Daisy×Round Pinkeye. | 5 | 150 | 30 | ...do... | 58 | .140 | .086 | .031 | .094 | .062 | .094 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 78 | | 4046 | Prof. Maerker ×Silverskin. | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | .109 | .078 | .659 | .304 | .047 | .140 | ..... | ..... | ..... | ..... | ..... | ..... |

[a] Leuco base of malachite green.

OXIDASE ACTIVITY OF THE JUICE OF THE SHOOTS

The procedure was very much like that used with healthy potato plants. The first experiments were carried out on the whole shoot of the plants. The results are summarized in Table IX.

There is no definite tendency observable in these results. The diluting influence of the stems is apparently more than compensated for by the

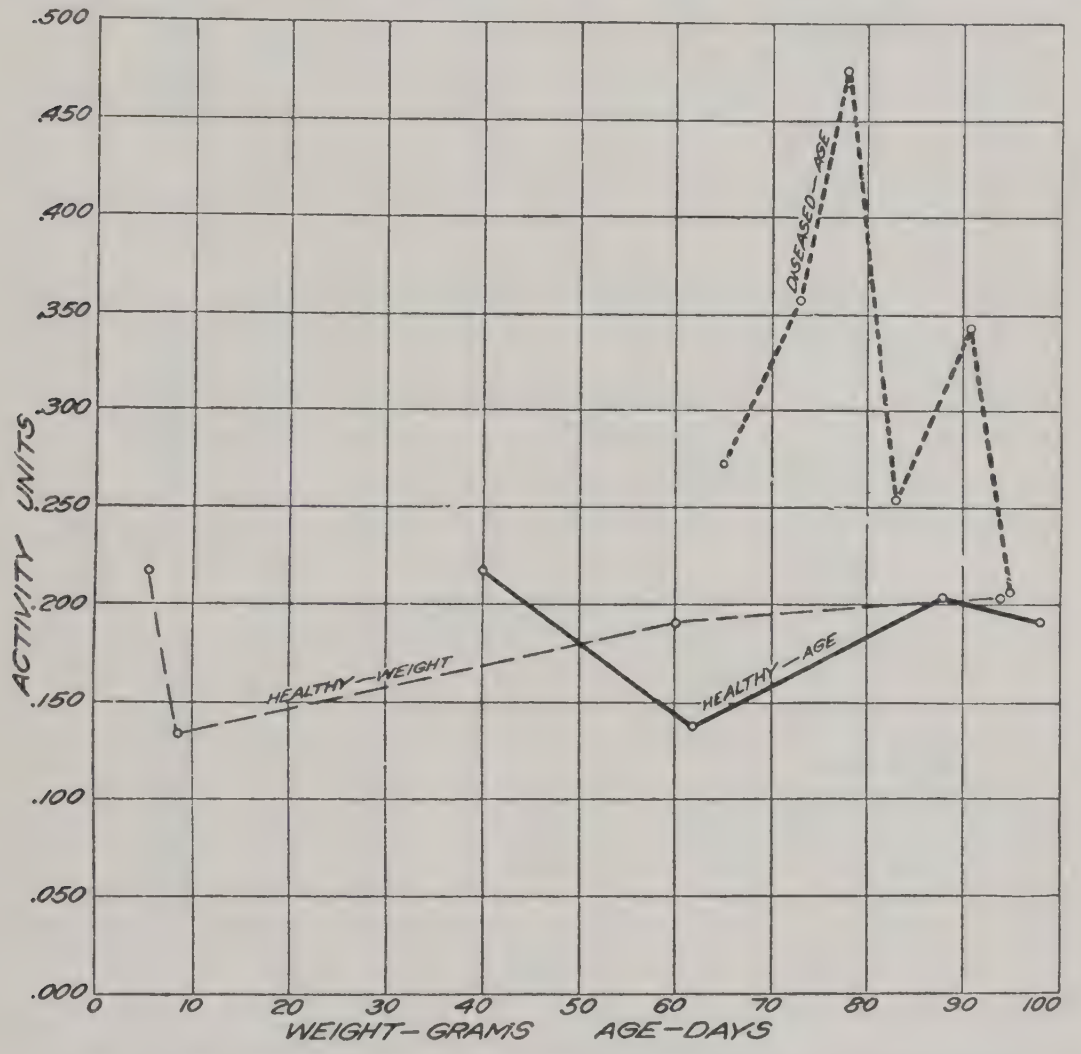



FIG. 13.—Curve showing oxidation of pyrogallol in the presence of the juice of potato tubers.

relatively higher oxidase content of the foliage of the older plants. All of the plants were started at the same time, so that from the viewpoint of age the results are comparable. As is shown by Table IX, no direct correlation can be found between the age or weight of the shoot and the oxidase content. These results are further discussed, together with other data on diseased foliage, on page 399.

OXIDASE ACTIVITY OF THE JUICE OF THE LEAVES

The results obtained in working with the foliage of curly-dwarf potato plants rather than with the whole shoots are given in Table X.

TABLE X.—*Oxidase activities of the juice of the leaves of curly-dwarf potato plants*

| Series No. | Date. | No. of variety. | Number of hills used. | Total weight of shoots. | Mean total weight of shoots per hill. | Number of shoots. | Mean weight of shoots. | Date of planting. | Age of plant. | Total weight of stems. | Stems × 100 = total weight of shoots. | Oxidase activity expressed in units as measured in the oxidation of the reagents. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | O-cresol. | M-cresol. | P-cresol. | O-toluidin. | M-toluidin. | P-toluidin. |
| | | | | *Gms.* | *Gms.* | | *Gms.* | | *Days.* | *Gms.* | *Gms.* | | | | | | | | | | | | | | | | | | |
| 79 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | 0.070 | 0.059 | ..... | 0.234 | 0.062 | 0.078 | ..... | ..... | ..... | ..... | ..... | ..... |
| 80 | Aug. 5 | [b]14980 | 4 | 380 | 95 | 4 | 95 | June 2 | 64 | 178 | 47 | 0.062 | 0.039 | 0 | 0.047 | 0.055 | 0.070 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 81 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 82 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.031 | 0.211 | 0.371 | 0.031 | 0.055 | 0.078 |
| 83 | Aug. 6 | [c]5014 [d]3438 | 3 | 440 | 147 | 12 | 37 | ...do.... | 65 | 254 | 58 | .109 | ..... | .047 | .086 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 84 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | .078 | .062 | ..... | .250 | .039 | .055 | ..... | ..... | ..... | ..... | ..... | ..... |
| 85 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .047 | .265 | .398 | .020 | .047 | ..... |
| 86 | | | | | | | | | | | | ..... | .062 | ..... | .016 | .027 | .062 | ..... | ..... | 0.374 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .062 |
| 87 | Aug. 7 | ..... | 3 | 530 | 177 | 14 | 38 | ...do.... | 66 | 284 | 54 | .031 | .035 | 0 | .031 | .008 | .035 | .016 | .055 | .367 | .226 | .027 | .078 | ..... | ..... | ..... | ..... | ..... | ..... |
| 88 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 89 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .047 | .226 | .320 | .031 | .035 | .023 |
| 90 | Aug. 14 | [e]2203 | 4 | 642 | 161 | 15 | 43 | ...do.... | 73 | 315 | 49 | .101 | .043 | .016 | .055 | .051 | .059 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 91 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | .117 | .047 | .585 | .261 | .055 | .137 | ..... | ..... | ..... | ..... | ..... | ..... |
| 92 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .047 | .312 | .460 | .078 | .140 | .055 |
| 93 | Aug. 19 | ..... | 4 | 500 | 125 | 20 | 25 | ...do.... | 78 | 198 | 40 | .039 | .016 | 0 | .039 | .016 | .031 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 94 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | .101 | .043 | .406 | .296 | 0 | .105 | ..... | ..... | ..... | ..... | ..... | ..... |
| 95 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .047 | .201 | .390 | .043 | .031 | .055 |
| 96 | Aug. 25 | [f]6061 | 2 | 890 | 445 | 5 | 178 | ...do.... | 84 | 440 | 50 | .070 | ..... | 0 | .062 | .051 | .078 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 97 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | .078 | .035 | .515 | .218 | .055 | .086 | ..... | ..... | ..... | ..... | ..... | ..... |
| 98 | Aug. 27 | [g]23457 | 5 | 550 | 110 | 7 | 79 | ...do.... | 86 | 210 | 38 | ..... | ..... | ..... | ..... | ..... | ..... | .078 | .098 | ..... | .347 | ..... | .125 | .047 | ..... | .410 | .062 | .101 | .078 |
| 99 | | | | | | | | | | | | .016 | (?).047 | .113 | .121 | .105 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 100 | Sept. 1 | [h]18743 | 4 | 440 | 110 | 7 | 63 | ...do.... | 91 | 180 | 47 | ..... | ..... | ..... | ..... | ..... | ..... | .148 | .117 | ..... | .324 | .086 | .156 | ..... | ..... | ..... | ..... | ..... | ..... |
| 101 | | | | | | | | | | | | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .086 | .464 | .554 | ..... | ..... | ..... |

[a] Leuco base of malachite green.
[b] Keeper×Silverskin.
[c] Pres. Kruger×Keeper.
[d] Prof. Maerker×Apollo.
[e] Sophie×Keeper.
[f] Daisy×Keeper.
[g] Early Eureka×Keeper.
[h] S. demissum×Keeper.

In order to ascertain whether the oxidase content of the leaves of these abnormal plants showed with age similar fluctuations to those found in healthy plants, curves representing the measurements of these oxidases were plotted with the curves representing the oxidase measure-

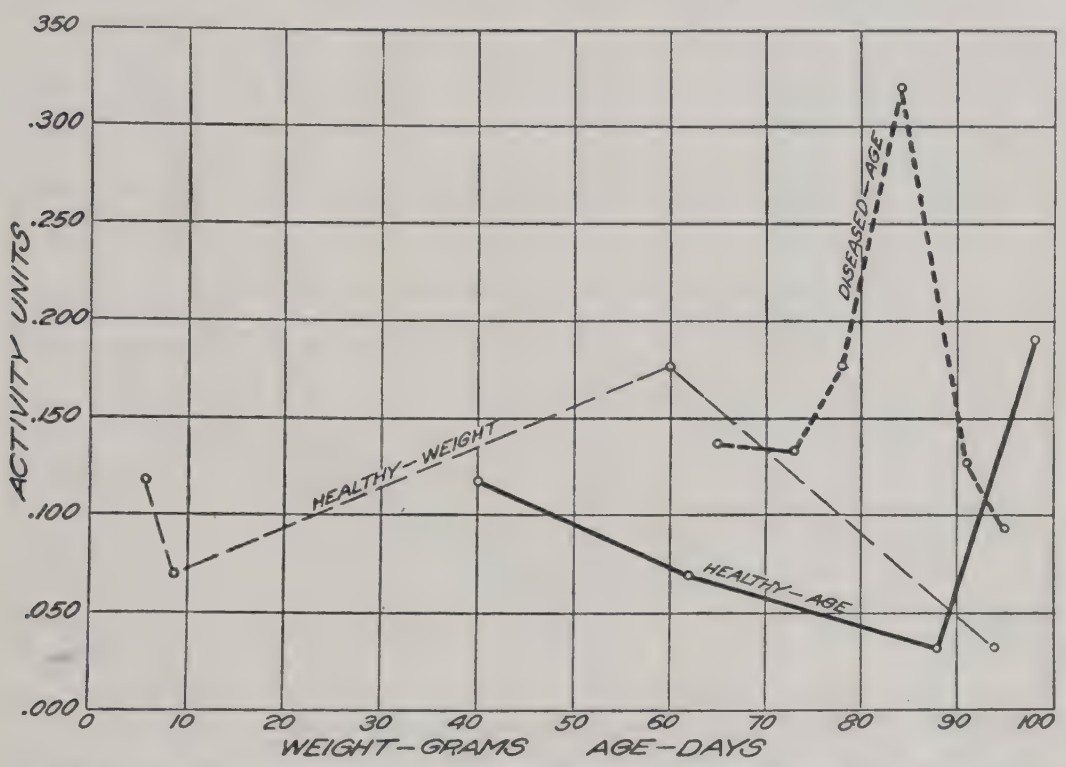



FIG. 14.—Curve showing oxidation of α-naphthol in the presence of the juice of potato tubers.

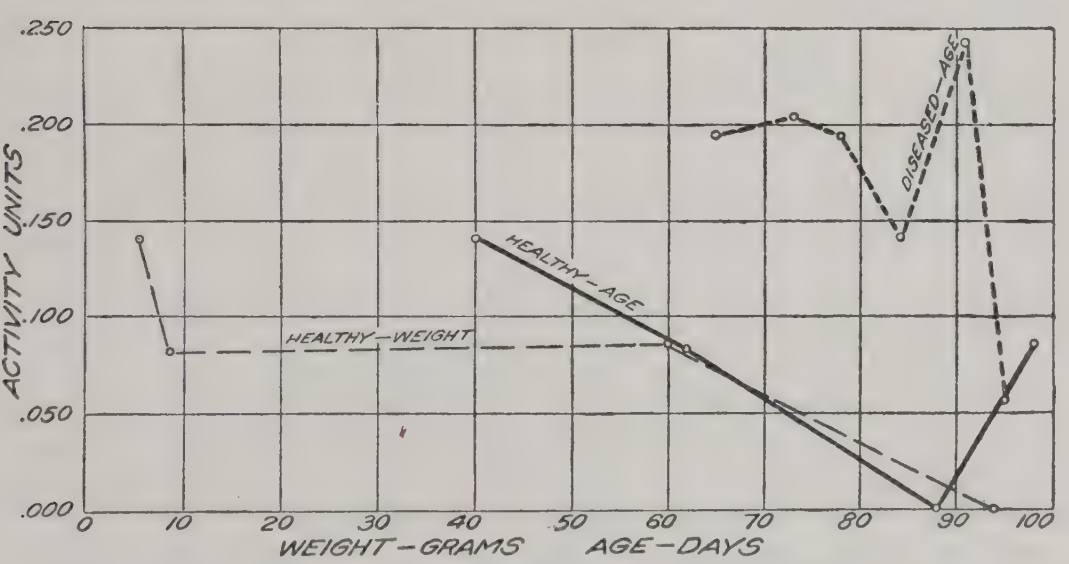



FIG. 15.—Curve showing oxidation of tyrosin in the presence of the juice of potato tubers.

ments of the healthy plants. Some of these are shown by the broken lines in figures 6 to 12. Plotting both curves on the same system of coordinates has the additional advantage of making possible a quick observation of the comparative magnitudes of oxidase activity in the healthy and diseased juices.

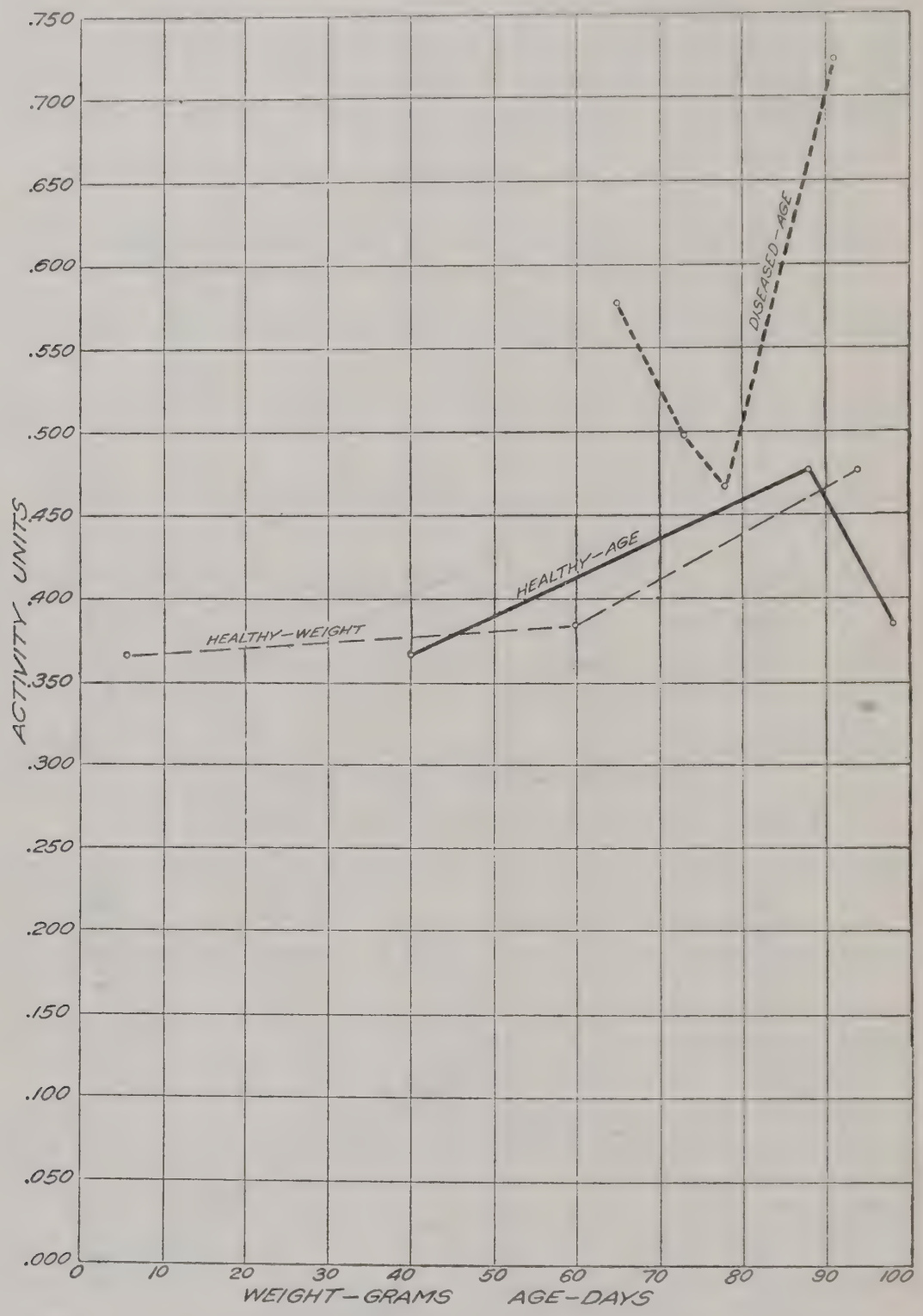



FIG. 16.—Curve showing oxidation of hydrochinone in the presence of the juice of potato tubers.

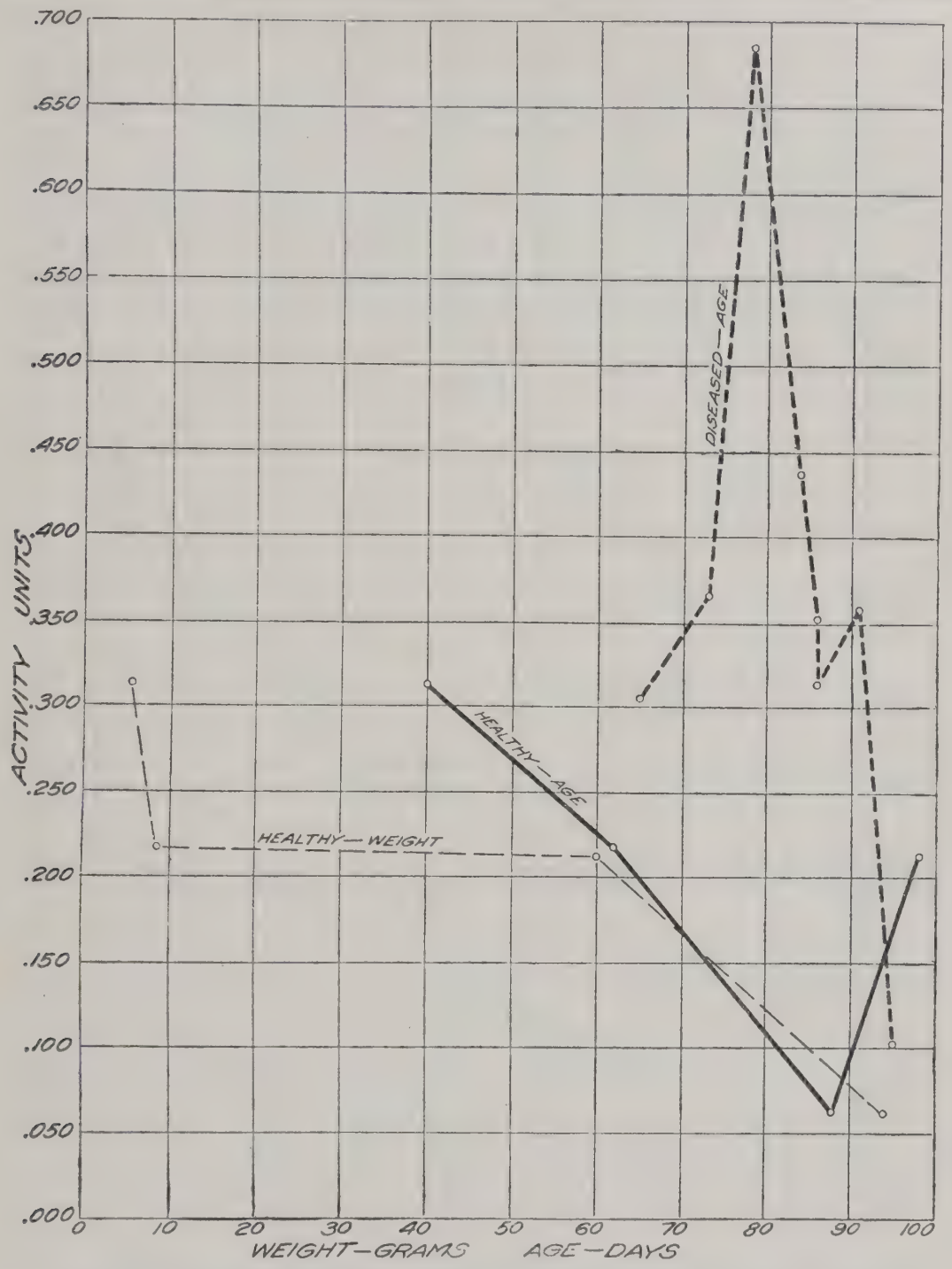



FIG. 17.—Curve showing oxidation of guaiacol in the presence of the juice of potato tubers.

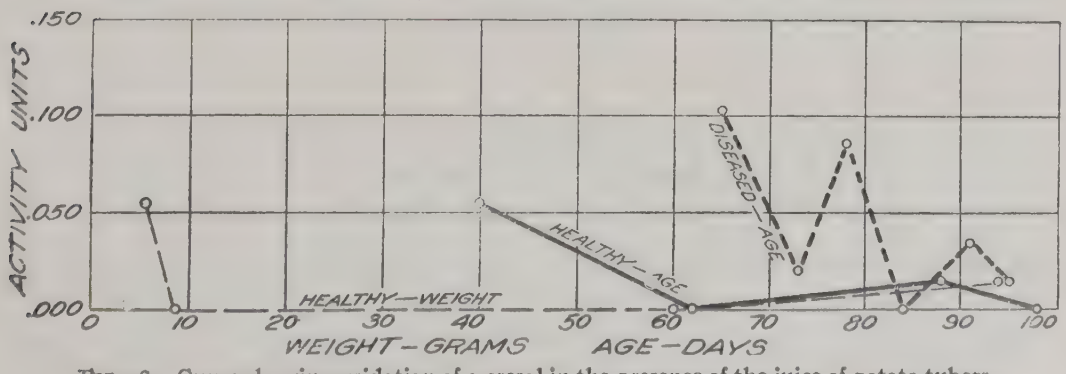



FIG. 18.—Curve showing oxidation of o-cresol in the presence of the juice of potato tubers.

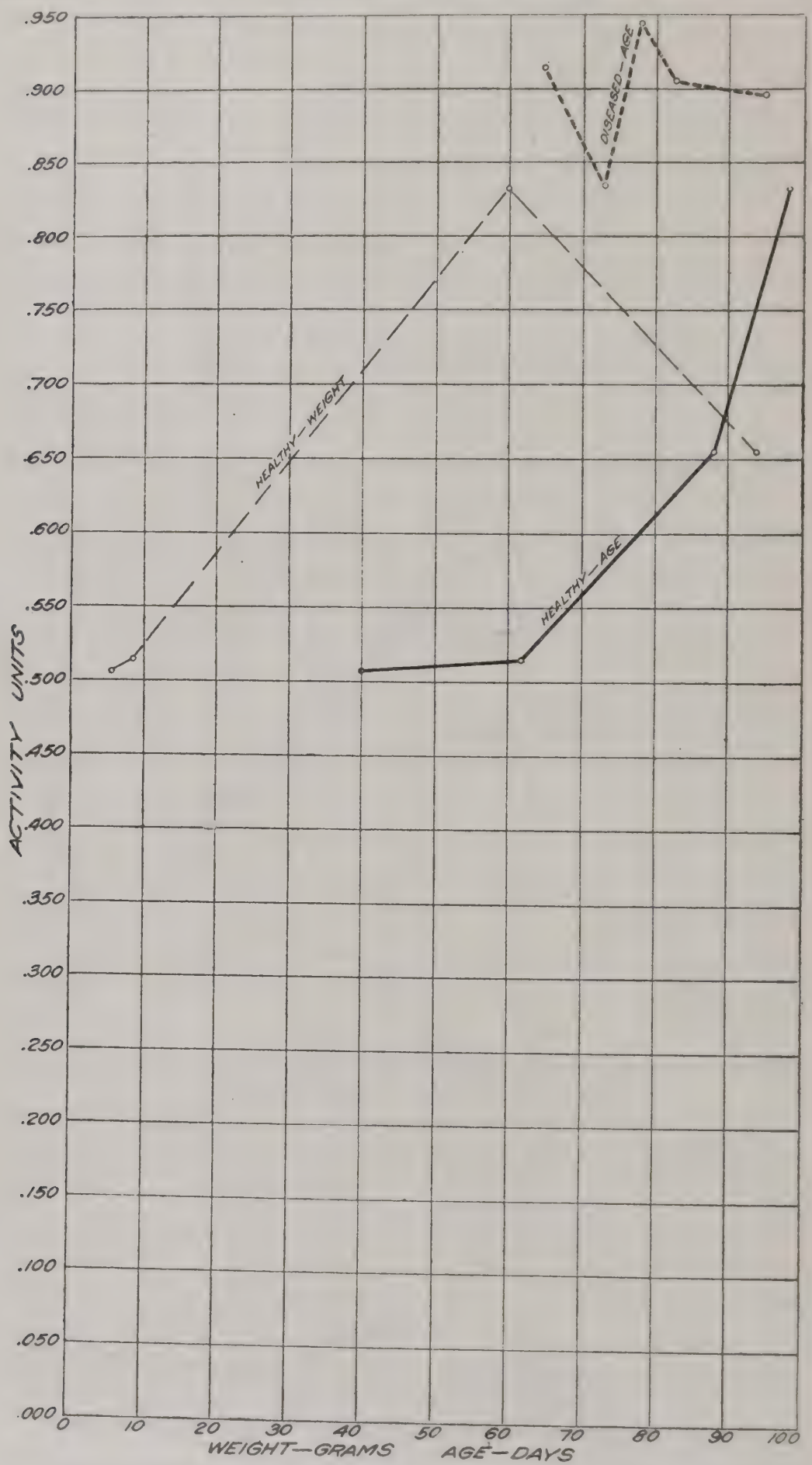



FIG. 19.—Curve showing oxidation of p-cresol in the presence of the juice of potato tubers.

These curves seem to show no definite tendency such as was seen in the case of the growing leaves of healthy plants. This is to be expected when it is considered that the physiological condition of the plants and presumably the oxidase contents of the juices are here influenced by two factors combined, age and disease. Past experience has shown that the oxidase activity of the plant juices is markedly affected by physiological disturbances such as the curly-dwarf disease of potatoes seems to be. The magnitude of the effect on the oxidase activities probably depends on such factors as the age of the plant when the diesase first took hold,

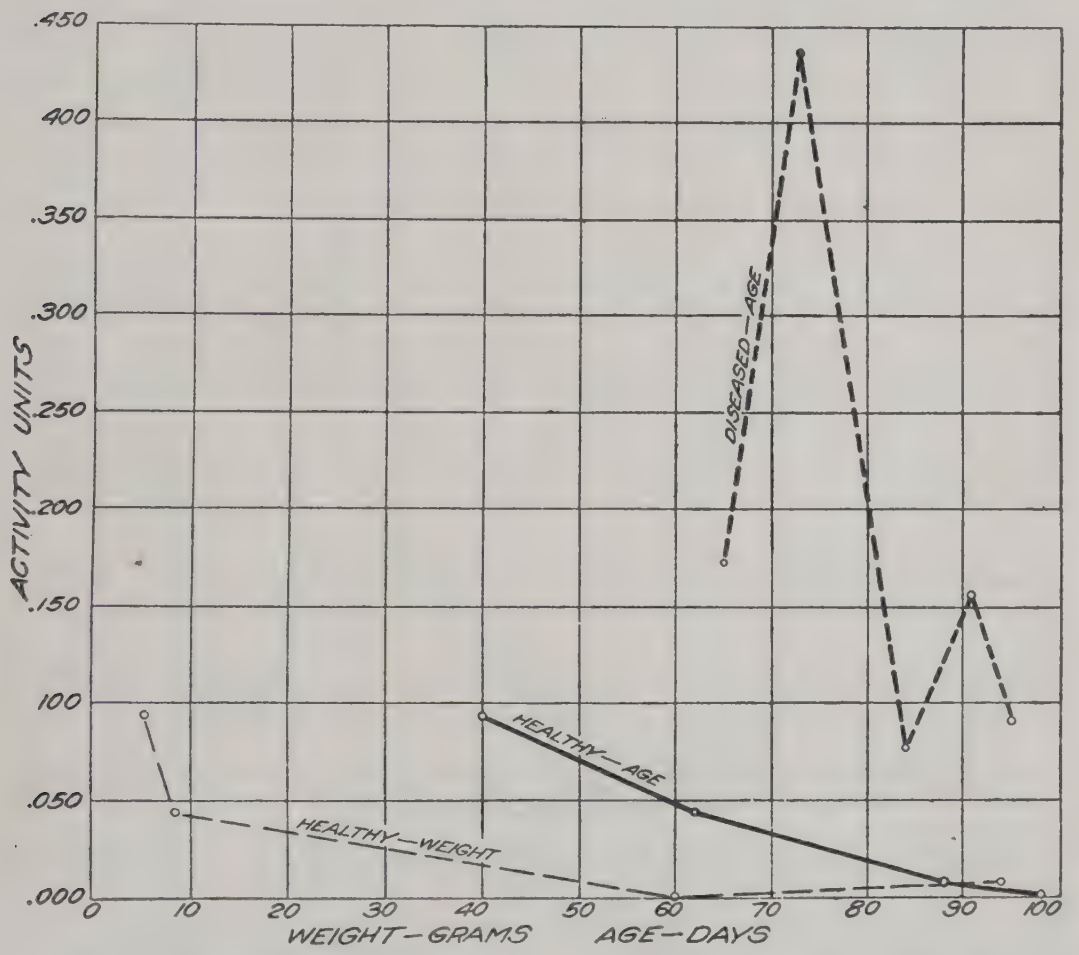



FIG. 20.—Curve showing oxidation of p-toluidin in the presence of the juice of potato tubers.

the length of time elapsed since, the individual resistance of the plant, etc. None of the factors are susceptible of measurement; the plants examined are influenced by them to different degrees and are therefore not comparable.

However, they all showed the typical curly-dwarf symptoms, and if the oxidase activities of the leaf juice are influenced by the apparent physiological disturbances the influence should be noticeable by a deviation of the oxidase activities from the normal and in a definite direction. That such a deviation from the normal actually exists is indicated by the curves in figures 6 to 12. With most of the reagents used the broken

lines, representing the oxidase activities of the curly-dwarf foliage, run at a higher mean level than the continuous lines, which represent the oxidase activities of the healthy foliage. The differences will be brought out in a mathematical form in a latter part of this paper.

To get a clear idea of the striking differences in the rate and extent of growth existing between the healthy and the diseased plants, the results showing these differences are represented graphically in figure 21. The ages of the plants are represented on the abscissæ, the mean weight

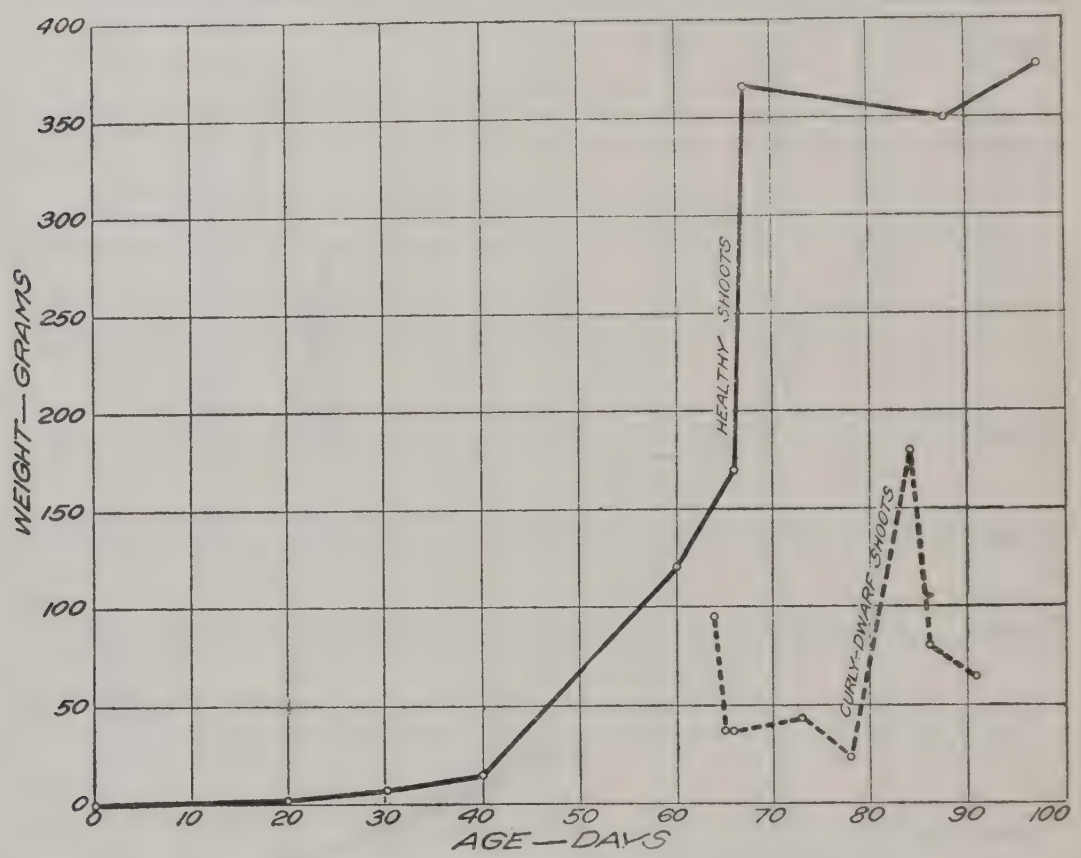



FIG. 21.—Curve showing the differences in rate and extent of growth between healthy and diseased plants.

of the shoots on the ordinates. The continuous line represents the growth of the healthy plants, the broken line that of the diseased ones.

The difference in weight of the two types of potato plants of the same age is strikingly apparent. The diseased plants made an average growth of only about one-eighth of the growth of the normal plants.

The fluctuations in the curve representing the rate of growth of curly-dwarf shoots are to be expected when the complex nature of the disease is considered. The diseased plants used for experimentation, although showing the typical symptoms, differed greatly in size, as is shown in figure 21, in color, and also in the shape which they assumed on account of the inhibition of growth.

### OXIDASE ACTIVITY OF THE JUICE OF THE TUBERS

The collection of the tubers of the diseased plants and the determinations of the oxidase content of the juices obtained from them were carried out in the same way as was done in the case of the healthy material. The results are summarized in Table XI and are included in figures 13 to 20. As before, the heavy broken lines represent the results obtained with curly-dwarf material.

These results, like those obtained with the tubers of healthy potato plants, show no definite tendency. If with age there is a definite variation in oxidative capacity exhibited toward all of the reagents, it is entirely masked by the irregular fluctuations. These irregular fluctuations were also observed in the case of diseased foliage and are illustrated in figures 6 to 12.

### DISCUSSION OF RESULTS

Comparison of the curves of the healthy plants with those of the diseased ones shows at a glance a greater oxidase activity in the case of the curly-dwarf material. This is true for both the tubers and the foliage. It seemed desirable to express these differences in some numerical form, and this was done by taking the averages of all the results obtained from material of the same type with the same reagents. These averages were then easily compared.

It was shown that healthy foliage yields juices of diminishing oxidase activity from the time of sprouting up to about the fortieth day of growth (as counted from the time of planting). For this reason in this summary of averages must be included only those of the results obtained with healthy leaves which were obtained during the growth periods of the diseased material examined. The age of the diseased foliage collected ranged from 64 to 91 days; the age of the plants where the whole shoots were examined was from 45 to 58 days. The averages were calculated as follows: All of the data (oxidase activities) obtained within the age periods mentioned were added together with the figures obtained for the beginning and the end of the period by interpolation from the curve. The sum, of course, was divided by the number of data added. These averages are shown in Table XII.

TABLE XI.—*Oxidase activities of the juice of potato tubers*

| Series No. | Series No. of leaves of same. | Date of collection. | Date of experiment. | Number of hills used. | Total number of shoots in same. | Total number of tubers. | Number of tubers per hill. | Number of tubers per shoot. | Total weight of tubers. | Mean weight of tubers. | Age of plant. | Oxidase activity of juice of tubers expressed in units using the reagents. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Benzidin. | Pyrogallol. | α-naphthol. | L. b. of m. g.[a] | Phloroglucin. | Aloin. | Pyrocatechol. | Tyrosin. | Hydrochinone. | Phloridzin. | Resorcin. | Guaiacol. | O-cresol. | M-cresol. | P-cresol. | O-toluidin. | M-toluidin. | P-toluidin. |
| | | | | | | | | | *Gms,* | *Gms,* | *Days.* | | | | | | | | | | | | | | | | | | |
| 102 | | | | | | | | | | | | 0.413 | 0.273 | 0.137 | 0.059 | 0.035 | 0.047 | | | | | | | | | | | | |
| 103 | 82 | Aug. 6 | Aug. 12 | 3 | 12 | 11 | 3.7 | 0.9 | 245 | 22.3 | 65 | | | | | | | 0.425 | 0.195 | 0.577 | 0.211 | 0 | 0.304 | | | | | | |
| 104 | | | | | | | | | | | | | | | | | | | | | | | | 0.101 | 0.647 | 0.913 | 0.004 | 0.031 | 0.172 |
| 105 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 106 | 89 | Aug. 14 | Aug. 15 | 4 | 15 | 27 | 6.8 | 1.8 | 175 | 6.5 | 73 | | .359 | .133 | .031 | .023 | .023 | | | | | | | | | | | | |
| 107 | | | | | | | | | | | | | | | | | | .082 | .203 | .499 | .234 | .008 | .367 | | | | | | |
| 108 | | | | | | | | | | | | | | | | | | | | | | | | .020 | .515 | .835 | 0 | .031 | |
| 109 | 92 | Aug. 19 | Aug. 20 | 4 | 20 | 94 | 23.5 | 4.7 | 365 | 3.9 | 78 | | .468 | .179 | .023 | | .070 | | | | | | | | | | | | |
| 110 | | | | | | | | | | | | | | | | | | .472 | .195 | .468 | .257 | | .686 | | | | | | |
| 111 | | | | | | | | | | | | | | | | | | | | | | | | .086 | .593 | .944 | .008 | | .437 |
| 112 | 95 | Aug. 25 | Aug. 26 | 2 | 5 | 15 | 7.5 | 3.0 | 530 | 35.3 | 84 | .250 | .254 | .320 | 0 | .031 | 0 | | | | | | | | | | | | |
| 113 | | | | | | | | | | | | | | | | | | .281 | .140 | | .289 | 0 | .437 | | | | | | |
| 114 | 98 | Aug. 27 | Aug. 28 | 5 | 7 | 14 | 2.8 | 2.0 | 505 | 36.0 | 86 | | | | | | | | | | | | | 0 | .218 | .905 | 0 | 0 | .078 |
| 115 | | | | | | | | | | | | | | | | | | | | | .265 | | .312 | | | | | | |
| 116 | | | | | | | | | | | | | | | | | | | | | .234 | 0 | .351 | | | | | | |
| 117 | 99 | Sept. 1 | Sept. 2 | 4 | 7 | 17 | 4.3 | 2.4 | 265 | 15.6 | 91 | .086 | .343 | .129 | 0 | .023 | .008 | | | | | | | | | | | | |
| 118 | | | | | | | | | | | | | | | | | | .078 | .242 | .722 | .254 | 0 | .359 | | | | | | |
| 119 | | | | | | | | | | | | | | | | | | | | | | | | .035 | .460 | | 0 | 0 | .156 |
| 120 | (b) | Sept. 5 | Sept. 6 | 3 | .... | 33 | 11 | ... | 590 | 17.9 | 95 | .176 | .207 | .094 | .016 | .031 | .043 | | | | | | | | | | | | |
| 121 | | | | | | | | | | | | | | | | | | | .059 | | .195 | .016 | .101 | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | .016 | | .897 | 0 | 0 | .090 |

[a] Leuco base of malachite green.

[b] Foliage dead.

TABLE XII.—*Relative oxidase activity of healthy and curly-dwarf-diseased potatoes*

| Reagent used. | Oxidase activity[a] of juices of— | | | | | | Difference in activity between juices of— | | |
|---|---|---|---|---|---|---|---|---|---|
| | Shoots of healthy plants collected at the age of 45 to 58 days. | Shoots of curly-dwarf plants collected at the age of 45 to 58 days. | Foliage of healthy plants collected at the age of 64 to 91 days. | Foliage of curly-dwarf plants collected at the age of 64 to 91 days. | Tubers of healthy plants. | Tubers of curly-dwarf plants. | Curly-dwarf and healthy shoots. | Curly-dwarf and healthy foliage. | Curly-dwarf and healthy tubers. |
| | | | | | | | *Per cent.* | *Per cent.* | *Per cent.* |
| Benzidin | 0.011 | 0.085 | 0.062 | 0.061 | 0.188 | 0.231 | + 673 | − 2 | + 23 |
| Pyrogallol | .002 | .053 | .026 | .040 | .187 | .318 | +2,550 | + 54 | + 70 |
| α-naphthol | .011 | .027 | .008 | .025 | .098 | .165 | + 145 | +213 | + 68 |
| Leuco base of malachite green | .014 | .043 | .040 | .057 | .015 | .022 | + 207 | + 42 | + 47 |
| Phloroglucin | .017 | .034 | .045 | .045 | .023 | .029 | + 100 | 0 | + 26 |
| Aloin | .013 | .048 | .065 | .056 | .023 | .032 | + 269 | − 14 | + 39 |
| Pyrocatechol | .027 | .076 | .056 | .086 | .261 | .268 | + 181 | + 54 | + 3 |
| Tyrosin | .016 | .057 | .068 | .064 | .077 | .172 | + 256 | − 6 | +123 |
| Hydrochinone | .312 | .450 | .382 | .450 | .409 | .567 | + 44 | + 18 | + 39 |
| Phloridzin | .112 | .272 | .106 | .269 | .248 | .243 | + 143 | +154 | − 2 |
| Resorcin | .023 | .042 | .058 | .046 | .016 | .004 | + 83 | − 21 | − 75 |
| Guaiacol | .047 | .103 | .079 | .102 | .200 | .364 | + 119 | + 29 | + 82 |
| O-cresol | .041 | .031 | .045 | .050 | .018 | .043 | − 24 | + 11 | +139 |
| M-cresol | .169 | .226 | .108 | .290 | .388 | .487 | + 34 | +168 | + 25 |
| P-cresol | .266 | .414 | .203 | .414 | .627 | .919 | + 56 | +103 | + 47 |
| O-toluidin | .036 | .024 | .057 | .044 | 0 | .002 | − 33 | − 23 | ..... |
| M-toluidin | .040 | .029 | .060 | .068 | .008 | .012 | − 28 | + 13 | + 50 |
| P-toluidin | .028 | .065 | .052 | .059 | .036 | .187 | + 132 | + 13 | +419 |

[a] Activity expressed in units as measured in the oxidation of the reagents.

As Table XII shows, the differences existing between the oxidase activity of the healthy and of the diseased material are generally marked and the greater activity is in the curly-dwarf potato plants. The comparison of the data for healthy and curly-dwarf shoots shows that among the 18 reagents only 3 are oxidized more readily in the presence of the juice of the healthy plants. Comparison of the leaves of the two types of plants shows 7 of the 18 reagents to be more readily oxidized by the healthy juice; in the case of the two types of tubers only two of the reagents showed greater oxidation by the healthy material. Among 54 sets compared, 12 showed a greater activity in the case of the healthy material, while the remainder, 42, showed a much greater activity in the case of the diseased plants.

It seems safe to conclude that in general the oxidizing power in the juices of curly-dwarf potato plants is greater than in those of healthy plants. The writer does not know as yet exactly what bearing, if any, the oxidases measured by him have on the oxidation processes going on in the cells. A priori, one would conclude that the intensity of oxidation processes in the cells would among other factors depend on the concentration of the various oxidases present. If this were the case, one would expect cell respiration to be more intense in the cells of the curly-dwarf tubers. The diseased plants would be in a condition corresponding to "fever" in animals.

These results agree in their general nature with those obtained in the case of the curly-top of sugar beets (Bunzel, 1913a, 1913b) and the leaf-roll of potatoes (Doby, 1911–12). In all three cases an increase in oxidases and a general retardation of growth are found. It would be extremely interesting, especially to plant physiologists, to find out what the rate of respiration is in such dwarfed, presumably "feverish" plants. Experiments intended to throw light on this point are already being planned in the laboratory of the Office of Plant Physiological and Fermentation Investigations.

There are a number of facts brought out in this investigation which open doors to new aspects of the physiology of development. There seems to be a cycle in the activity of the expressed juice of the foliage of normally developing potato plants. The juice of the foliage of very young plants is more active than that of plants of the same variety 40 or 50 days older; after that stage of development the activity rises again with increasing age. Quite in harmony with these findings is the fact that sprouts of artificially sprouted tubers of the same variety are much more active than the youngest foliage examined.

There seems to be a parallelism, therefore, between the intensity of physiological activity and the quantity of oxidases present. This belief is strongly corroborated by the fact that the physiologically more active portions of the plant, such as the leaves, furnish juices with greater activity than the obviously less active portions of the same plant, such as

the stems. This has been found by the writer not only in the case of the potato plants, but also in sugar beets (Bunzel, 1913a, 1913b).

In this connection the results obtained by Nicolas (1907) are very interesting. He studied the respiration of individual parts of plants and found that those organs which carry out the assimilating functions of the plant show the greatest respiratory activity. The limbs or the organs which replace them in function, such as the phyllodia or cladodia, have 1.4 to 4.5 times as great a respiratory activity as the petiole, stem, or tendrils. These results when combined with those obtained by the writer in the present investigation would indicate that there is at least a general parallelism between the oxidase activity of the juice obtained from a plant organ and the intensity of its physiological activity, as measured by its intensity of respiration. Plans are made to study the question more closely in the laboratory of the Office of Plant Physiological and Fermentation Investigations.

## SUMMARY

(1) The oxidase activity of the foliage of normally developing potato plants is greatest in the early stages of development; it falls off with growth of the plants and rises again when the plant's growth about reaches a standstill.

(2) Curly-dwarf potato plants show a greater oxidase activity than healthy ones of the same age, both in the juice of their tubers and in the juice of their foliage.

(3) The oxidative activity of the different parts of the potato plant has been established for 18 different reagents.

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# BIRDS AS CARRIERS OF THE CHESTNUT-BLIGHT FUNGUS[1]

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## INTRODUCTION

Various writers have expressed the opinion that birds play a part in the dissemination of the chestnut-blight fungus, *Endothia parasitica* (Murr.) And. Since most of these statements were not based on any published investigations, the work described in this paper (6)[2] was undertaken in order to furnish positive evidence as to whether birds actually do carry spores of this fungus.

Birds do not seem to have been extensively accused of spreading plant diseases. Evidence has been presented by Waite (15) that pear-blight is disseminated by humming birds. The same writer (16) also gives a brief statement of the part played by sapsuckers and brings out the probability of long-distance distribution of blight germs by birds. It has been stated (7) that the organism causing the olive-knot disease is carried by birds. Johnston (8) has expressed the belief, based upon some experiments which he conducted, that turkey buzzards are instrumental in spreading the bud-rot of the coconut. In all of these instances the causal organisms are bacteria. Only a single reference has come to our attention in which birds have been charged with spreading fungous diseases, except in the case of the chestnut blight, as will be stated later. In a consideration of the die-back (Naemospora) of peaches, Massee (10, p. 449) says that "probably the conidia are conveyed on the feet of birds from diseased to healthy shoots."

## HISTORICAL REVIEW

The first article published by Murrill (13) on chestnut blight refers to the possible relation of birds to the dissemination of the disease, as follows: "And from these numerous yellowish brown pustules millions of

[1] Investigations conducted in cooperation with the Pennsylvania Chestnut Tree Blight Commission.
[2] Bibliographic citations in parentheses refer to "Literature cited," pp. 421–422.

minute summer spores emerge from day to day in elongated reddish brown masses to be disseminated by the wind and other agencies, such as insects, birds, squirrels, etc." The same author also says (13) "every bird and insect that rests upon an infected spot is liable to carry the spores upon its feet or body to other trees." A few years later Mickleborough (12) mentions birds as carriers of spores of the blight fungus. He says "the minute spores are carried by wind, on the feathers of birds, and the fur of squirrels." Still later Metcalf and Collins (11) say that "there is strong evidence that the spores are spread extensively by birds, especially woodpeckers." Various writers have mentioned the fact that woodpeckers frequent chestnut trees in search of insects. Fulton (2) states in a report on field work done at Orbisonia, Pa., by Mr. R. C. Walton that "woodpecker work was noted in about one-tenth of the oldest lesions," but he offers no conjecture as to the part played by birds in the dissemination of the disease.

Stewart (14) says that "undoubtedly the spores are carried long distances by birds, especially woodpeckers, which visit the diseased trees, seeking borers, in the tunnels of which most of the infections occur." This statement is based on the report of Metcalf and Collins previously referred to and is discredited by Fisher (1), who brings out the point that this and similar statements are not based on positive evidence.

Kittredge (9) reports that field observations at Petersham, Mass., indicate "that birds may be a very important, if not the primary agent," in the distribution of the blight fungus. He is led to this conclusion from the greater number of infections near the borders of coniferous woods, where, he says, birds are more abundant, and from the much larger number of lesions in the middle third of the trunk, which he attributes to the work of creepers, nuthatches, and woodpeckers.

There are numerous popular articles which also accuse birds of being instrumental in the spread of the blight, but these, as well as the statements already quoted, are based entirely on circumstantial evidence.

The first serious attempt to determine whether birds actually do carry the spores of the chestnut-blight fungus was made by the field pathologists of the Pennsylvania Chestnut Tree Blight Commission during the summer of 1912. Since only negative results were obtained, it may be well to quote their statement giving the method employed:

> Birds found on the infected parts of trees were shot during the summer, and their feet, bills, and tail feathers washed separately in sterile water. This water was then centrifuged to bring down the spores that might have been washed from the birds. Part of the sediment was then examined under the microscope and the other part plated out in dilution plates. When colonies of fungi appeared, they were isolated to determine whether they were Diaporthe.

The above description is not sufficiently detailed to make possible an accurate judgment as to whether the negative results obtained were due to

imperfect technique, but our experience leads us to believe that such may have been the case. Plates heavily seeded with bacteria and various fungi give no accurate or reliable results, since the colonies of the chestnut-blight fungus are very slow growing and would be overrun before they had reached sufficient size to be visible to the naked eye. Pycnospore colonies of this fungus at ordinary laboratory temperatures are barely large enough to mark at the end of four days and so would be entirely overlooked in plates crowded with bacteria and other fungi (3).

The negative results reported were based upon analyses of the following: Downy woodpeckers, 8; creepers (kind not specified), 3; hairy woodpeckers, 2; flickers, 4; bluejays, 3; total, 20.

## METHOD USED IN EXPERIMENTS

Nearly all the birds tested by the writers were shot [1] either at Martic Forge or at West Chester, Pa., or in the vicinity of these places, in order that use could be made of the rainfall and temperature records which were kept at these stations. The birds from Martic Forge were shot in or near a 300-acre orchard of badly diseased Paragon trees grafted on native stock. Those from West Chester were taken in the main from a young coppice growth which is practically 100 per cent diseased.

Most of the birds were shot from diseased trees, and in many cases they were working on cankers at the time of shooting or had been seen on chestnut-blight lesions a few minutes before they were killed. They were immediately placed in sterile paper sacks for transport to the laboratory at the University of Pennsylvania.

In the laboratory the procedure was, first, to sterilize a moist chamber in a Lautenschläger sterilizer for 35 minutes at 150° C. A stiff bristle brush was also sterilized in boiling water for half an hour or more. Before beginning work in the culture room the hands and arms of the operator were washed with soap and water and then in mercuric-chlorid solution (1 to 1,000).

When the moist chamber had cooled to room temperature, a flask containing 100 c. c. of sterile tap water was emptied into it. The bird to be tested was held in one hand and the feet, wing, and tail feathers and the head and the bill scrubbed vigorously with the brush, the operation being carried out so that only the parts scrubbed were permitted to come in contact with the wash water. The moist chamber was then well shaken, so as to secure a uniform suspension, and 1 c. c. of the wash water was transferred with a sterile pipette to a second flask containing 99 c. c. of sterile tap water. With a second sterile pipette 1 c. c. or fractions were transferred from the second flask, which had also been well shaken, to each of a series of Petri dishes. The dilutions used varied

[1] The birds used in this work were shot by Mr. C. E. Taylor, formerly in the employ of the Pennsylvania Chestnut Tree Blight Commission, who also centrifuged the wash water for its sediment.

somewhat, but the following were found to give the most satisfactory results:

1 c. c. from second flask to each of 2 Petri dishes.
10 drops from second flask to each of 4 Petri dishes.
5 drops from second flask to each of 4 or 6 Petri dishes.
1 drop from second flask to each of 4 Petri dishes.

In this way the number of plates per bird ranged from 12 to 20 in all cases, except bird No. 1. The number of drops delivered per cubic centimeter by the pipette were counted each time and were found to be fairly constant for any single pipette, although they varied from 24 to 53 drops for the different ones used. A tube of melted 3 per cent dextrose agar plus 10, which had been previously cooled to from 42 to 45° C., was added to each Petri dish and the plates rotated, so as to secure a uniform distribution of the spores. The entire operation of scrubbing and plating was carried out in a culture room, with every precaution against contamination from any source.

The plates were incubated in an inverted position in the laboratory and an attempt was made to keep the temperature of the room as nearly as possible at 25° C. At the end of four days those colonies suspected of being the chestnut-blight fungus were marked with india ink (3). Two or three days later this diagnosis was verified, and all doubtful colonies were transferred to other agar plates, to make certain of their identity. A count was also made of the number of bacterial and yeast colonies, of the number of fungous colonies other than those of *Endothia parasitica*, and of the number of species of fungi represented, as nearly as could be determined from cultural characteristics. With this information and knowing the calibration of the pipette used, it was an easy matter to compute the total number of viable spores or bacteria carried by each bird.

The original wash water was poured back into the flask and several cubic centimeters of formalin added to inhibit the growth of the spores. At a later time the wash water of those birds yielding positive results was centrifuged in 10 c. c. quantities, the sediments thus obtained thrown together and centrifuged again, so that the entire sediment was concentrated in about 2 c. c. of water. This final sediment was given a thorough microscopic examination, primarily for its pycnospore or ascospore content of *Endothia parasitica* and secondarily for the number and kinds of other fungous spores which it might contain.

## LIST OF BIRDS TESTED

A detailed record of the place and time of shooting of each bird is given below.

The birds tested belonged to nine different species. (See Table I.) One of these, the flicker, gets most of its food from the ground, although, as a rule, it flies into a tree when the person approaching is still a good distance away. Another species, the junco, gets practically all of its

food from the ground; both juncos tested were, however, shot out of infected trees. The golden-crowned kinglet is found mostly among foliage. The six other species, the black-and-white creeper, the brown creeper, downy woodpecker, hairy woodpecker, sapsucker, and white-breasted nuthatch, are birds which are in the habit of creeping or climbing over the bark of the trunk and larger branches. As these were considered the most likely carriers of the spores of the chestnut-blight fungus, nearly all of the birds tested, 32 out of 36, belonged to these species. Particular attention was paid to the movements of the birds at the time of shooting, noting especially whether they were working on cankers or, at least, in blighted trees.

It is not uncommon, as stated before, to find evidence that woodpeckers have been at work in older lesions. An example of this is shown in Plate XXXVIII.

Bird No. 1.—Hairy woodpecker (*Dryobates villosus*). (0.)[1]

Shot at Tyrone, Pa., on December 11, 1912. Received and cultures made on December 12, 1912.

Bird No. 2.—Downy woodpecker (*Dryobates pubescens medianus*). (0.)

Shot at Martic Forge, Pa., at 1.10 p. m., on February 21, 1913, while at work on a canker about 12 feet from the ground. Bird was not killed at once, but fluttered along ground for 8 or 10 feet. Canker on which bird was working showed light-orange stromata, with abundant papillæ that were fairly prominent. Cultures made on February 22, 1913.

Bird No. 3.—Junco (*Junco hyemalis*). (0.)

Shot at Martic Forge, Pa., at 10.30 a. m., on February 28, 1913, from blight-infected chestnut tree. Rain of previous night was 0.36 inch, and the air was still very humid at the time the bird was taken. Plates made on March 1, 1913.

Bird No. 4.—Junco. (10,000.)

Same as Bird No. 3.

Bird No. 5.—Downy woodpecker. (30,000.)

Shot at Martic Forge, Pa., on March 10, 1913, out of a small tulip tree. Had been picking about some large, badly diseased, and dead chestnut trees. Cultures made on March 11, 1913.

Bird No. 6.—Downy woodpecker. (73,333.)

Shot near diseased coppice growth at West Chester, Pa., at 10.30 a. m., on March 19, 1913. Had been working on a small canker. Cultures made on March 19, 1913.

Bird No. 7.—Downy woodpecker. (109,022.)

Shot in diseased coppice growth, West Chester, Pa., at 12.30 p. m., on March 19, 1913. Had been working on a canker. Cultures made on March 19, 1913.

Bird No. 8.—Downy woodpecker. (92,000.)

Shot in diseased coppice growth at West Chester, Pa., at 12.40 p. m., on March 19, 1913. Was on a canker when shot. Received and cultures made on March 19, 1913.

Bird No. 9.—Flicker (*Colaptes auratus luteus*). (0.)

Shot at Martic Forge, Pa., on March 24, 1913. Came from the badly infected wood lot to the west of the orchard and was killed in the orchard. Received and cultures made on March 25, 1913.

[1] The numbers in parentheses, following the names of the birds, represent the number of spores of the chestnut-blight fungus carried, as determined by cultures. See Table I.

Bird No. 10.—Downy woodpecker. (757,074.)

Shot from a chestnut tree at Martic Forge, Pa., at 2 p. m., on March 28, 1913. Had been working on a canker, but was not on a canker when killed. Received and cultures made on March 29, 1913.

Bird No. 11.—Downy woodpecker. (15,625.)

Shot at Martic Forge, Pa., at 11 a. m., on March 31, 1913. Was on a canker on an old chestnut tree (22 inches D. B. H.) when killed. Received and cultures made on April 1, 1913.

Bird No. 12.—Downy woodpecker. (31,111.)

Shot at York Furnace, Pa. (near Martic Forge), at 11 a. m., on April 2, 1913. Had been working around a canker on a large chestnut tree. Received and cultures made on April 3, 1913.

Bird No. 13.—Downy woodpecker. (25,000.)

Shot at York Furnace, Pa., at 11.30 a. m., on April 2, 1913. Was on a canker on a large chestnut tree when killed. Received and cultures made on April 3, 1913.

Bird No. 14.—White-breasted nuthatch (*Sitta carolinensis*). (0).

Shot at York Furnace, Pa., at 1 p. m., on April 2, 1913. Shot out of a chestnut-oak tree; had not been seen on any chestnut trees. Received and cultures made on April 3, 1913.

Bird No. 15.—Downy woodpecker. (0.)

Shot at Martic Forge, Pa., at 10 a. m., on April 3, 1913. Was on chestnut tree when killed; had been drumming on an old tree. Not seen on cankers. Received and cultures made on April 4, 1913.

Bird No. 16.—Brown creeper (*Certhia familiaris americana*). (0.)

Shot at Martic Forge, Pa., at 12.30 p. m., on April 7, 1913, from chestnut tree to the side of which it was clinging. Trunk was apparently sound, and no cankers were visible from the ground. Received and cultures made on April 8, 1913.

Bird No. 17.—Golden-crowned kinglet (*Regulus satrapa*). (6,566.)

Shot at Martic Forge, Pa., at 12.30 p. m., on April 7, 1913, from a hemlock tree. Had been climbing up and down trees; was seen on oaks, but not on chestnut. Received and cultures made on April 8, 1913.

Bird No. 18.—Sapsucker (*Sphyrapicus varius*). (5,000.)

Shot from a hickory tree beyond Broomall, Pa., 9 miles west of Philadelphia, at 4 p. m., on April 10, 1913. Had been visiting cankers on chestnut trees. Received and plated out at 8.30 p. m. on April 10, 1913.

Bird No. 19.—White-breasted nuthatch. (5,655.)

Shot out of a chestnut tree beyond Broomall, Pa., at 4 p. m., on April 10, 1913. Had been running up and down trunk of chestnut tree and about cankers. Received and plated out at 8.30 p. m. on April 10, 1913.

Bird No. 20.—Downy woodpecker. (0.)

Shot out of a small dogwood tree about 5 miles west of Philadelphia, Pa., at 4 p. m., on April 10, 1913. Had been working around cankers. Received and plated out at 8.30 p. m. on April 10, 1913.

Bird No. 21.—Downy woodpecker. (5,780.)

Shot out of a soft-maple tree, 2 rods west of orchard at Martic Forge, Pa., on April 15, 1913. Had not been seen on or about any chestnut trees. Received and plated out on April 16, 1913.

Bird No. 22.—Sapsucker. (7,502.)

Shot out of a chestnut tree in a small grove immediately west of blighted orchard at Martic Forge, Pa., at 3.05 p. m., on April 17, 1913. Received and cultures made on April 18, 1913.

Bird No. 23.—Brown creeper. (254,019.)

Shot out of a black oak tree in woods north of coppice growth at West Chester, Pa., on April 18, 1913. Had not been seen on or about chestnut trees. Received and plated out on April 19, 1913.

Bird No. 24.—Black-and-white creeper (*Mniotilta varia*). (0.)

Shot out of a dogwood tree at Martic Forge, Pa., at 10.40 a. m., on April 21, 1913. Had been running up and down a chestnut tree, but was not seen on any cankers. Received and plated out on April 22, 1913.

Bird No. 25.—Downy woodpecker. (27,108.)

Shot while on a canker on a chestnut tree at West Chester, Pa., at 10.30 a. m., on April 25, 1913. Received and plated out on April 26, 1913.

Bird No. 26—Downy woodpecker. (59,742.)

Shot just east of chestnut orchard in a sprout growth of chestnut and oak at Martic Forge, Pa., at 12 m., on April 30, 1913. Had not been seen on or about cankers. Received and plated out on May 1, 1913.

Bird No. 27.—Downy woodpecker. (624,341.)

Shot just east of chestnut orchard from a canker in sprout growth at Martic Forge, Pa., at 12 m., on April 30, 1913. Received and plated out on May 1, 1913.

Bird No. 28.—Black-and-white creeper. (0.)

Shot out of a chestnut oak tree in woods north of sprout growth at West Chester, Pa., at 2.20 p. m., on May 2, 1913. Was not seen on or about any chestnut trees. Received and plated out on May 3, 1913.

Bird No. 29.—Hairy woodpecker. (0.)

Shot out of chestnut tree west of badly infected coppice at West Chester, Pa., on May 2, 1913. Was not positively seen on or about cankers. Received and plated out on May 3, 1913.

Bird No. 30.—Black-and-white creeper. (0.)

Shot in sprout growth of chestnut and oak east of the chestnut orchard at Martic Forge, Pa., at 10.15 a. m., on May 5, 1913. Was not seen on or about cankers. Received and plated out on May 6, 1913.

Bird No. 31.—Black-and-white creeper. (0.)

Shot from a badly diseased chestnut tree in sprout growth east of orchard at Martic Forge, Pa., at 11 a. m., on May 5, 1913. Was not seen on or about cankers. Received and plated out on May 6, 1913.

Bird No. 32.—Black-and-white creeper. (0.)

Shot in timber just west of orchard at Martic Forge, Pa., at 12.30 p. m., on May 5, 1913. Had been seen on chestnut trees, but not about cankers. Received and plated out on May 6, 1913.

Bird No. 33.—Downy woodpecker. (36, 312.)

Shot in wood lot east of coppice at West Chester, Pa., at 10 a. m., on May 9, 1913. Probably was on a canker when shot, but was too high up for this to be positively determined. Received and plated out on May 9, 1913.

Bird No. 34.—Black-and-white creeper. (0.)

Shot out of a diseased chestnut tree about which it had been creeping, north of diseased coppice at West Chester, Pa., at 10.30 a. m., on May 9, 1913. Received and plated out on May 9, 1913.

Bird No. 35.—Hairy woodpecker. (0.)

Shot from a dead chestnut stub west of diseased coppice at West Chester, Pa., at 12.55 p. m., on May 9, 1913. Was not seen on or about any cankers. Received and plated out on May 9, 1913.

Bird No. 36.—Black-and-white creeper. (0.)

Shot in orchard at Martic Forge at 10.30 a. m., on May 12, 1913. Had been creeping over a small canker. Received and plated out on May 13, 1913.

## RESULTS OF TESTS AS SHOWN BY CULTURES

The results obtained from the cultures are given in Table I.

Of the 36 birds tested 19 were found to be carrying spores of the chestnut-blight fungus. The highest positive results were obtained from two downy woodpeckers, which were found to be carrying 757,074 and 624,341 viable spores of *Endothia parasitica*. The next highest was a brown creeper, with 254,019 spores. In each case where the number of colonies of *Endothia parasitica* was very large, there was only a relatively small number of other fungous colonies present. This is best shown in bird No. 10, which yielded almost 14 times as many colonies of the chestnut-blight fungus as of all other fungi. Another good example, although not quite as striking, is bird No. 23, where the proportion was 5 to 1. Part of the plate cultures from bird No. 23 were photographed at the end of nine days (Pl. XXXIX). These show the characteristic development of *Endothia parasitica* colonies on dextrose agar and also the relatively small number of other fungous colonies present.

Positive results were obtained from one of the two juncos tested. Although this species is primarily ground-frequenting in habit, both juncos were shot from blight-infected trees. There are, therefore, two possible sources for the 10,000 spores of the chestnut-blight fungus carried by bird No. 4. First, the blighted tree from which it was shot; second, the pycnospores which had been washed into the soil around the bases of infected trees and which have been found to remain viable for a period of 2 to 13 days of dry weather (5).

In those cases in which the birds were shot directly from a chestnut-blight canker it might be suggested that the spores carried were scattered by the impact of the shot and lodged upon the feathers, and so were not obtained during the normal movements. A brief consideration of the tests of birds giving positive results will throw definite light on this subject.

Of the 19 birds yielding positive results only 6 were on cankers when killed, and positive results were also obtained from 6 of the 8 birds which had been working on cankers just previous to shooting, but which were not on cankers when killed. Again, 7 birds of the 20 which were not seen on cankers at all yielded positive results.

Of the 4 birds yielding the highest numbers of spores of the chestnut blight fungus, only 1, No. 27, was on a canker when shot; 2, Nos. 7 and 10, were not on cankers when shot but had been working on some previous to being killed; while 1, No. 23, had never been seen on a canker.

These results point clearly to the fact that the impact of the shot was not responsible for the presence or any increase in number of blight spores upon the bodies of the birds.

It will be noted that the number of bacterial and yeast colonies was quite large in most instances. The plates from five birds were so heavily

seeded with bacteria that it was impossible to get a reliable count of the number of fungous colonies, or even a test of the presence of colonies of *Endothia parasitica*. In most instances, however, the bacteria caused little or no trouble.

TABLE I.—*Results of tests of birds Nos. 1 to 36 as fungus carriers as shown by cultures*

| No. | Date. | Locality where shot (Pennsylvania). | Kind of bird. | Number of cultures. | Number of bacteria and yeasts. | Total number of fungous colonies. | Number of Endothia parasitica colonies. | Number of fungous species not Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | 1912. | | | | | | | |
| 1 | Dec. 11 | Tyrone..... | Hairy woodpecker. | 6 | ..... | ..... | ..... | ..... |
| | 1913. | | | | | | | |
| 2 | Feb. 21 | Martic Forge.. | Downy woodpecker. | 18 | 7,600,000 | 156,600 | 0 | 12 |
| 3 | Feb. 28 | .....do..... | Junco..... | 12 | 1,800,000 | 80,000 | 0 | 9 |
| 4 | ...do.... | .....do..... | .....do..... | 12 | 56,500 | 125,000 | 10,000 | 14 |
| 5 | Mar. 10 | .....do..... | Downy woodpecker. | 18 | 62,500 | 642,500 | 30,000 | 10 |
| 6 | Mar. 19 | West Chester.. | .....do..... | 12 | 950,000 | 173,333 | 73,333 | 6 |
| 7 | ...do.... | .....do..... | .....do..... | 12 | 127,819 | 173,648 | 109,022 | 7 |
| 8 | ...do.... | .....do..... | .....do..... | 12 | 76,000 | 328,000 | 92,000 | 10 |
| 9 | Mar. 24 | Martic Forge.. | Flicker..... | 12 | 12,910,000 | 60,000 | 0 | 10 |
| 10 | Mar. 28 | .....do..... | Downy woodpecker. | 18 | 69,827 | 812,164 | 757,074 | 6 |
| 11 | Mar. 31 | .....do..... | Downy woodpecker. | 18 | 190,625 | 262,500 | 15,625 | 11 |
| 12 | Apr. 2 | York Furnace. | .....do..... | 12 | 2,964,444 | 444,444 | 31,111 | 6 |
| 13 | ...do.... | .....do..... | .....do..... | 12 | 18,300,000 | 285,000 | 25,000 | 7 |
| 14 | ...do.... | .....do..... | White-breasted nuthatch. | 12 | 225,000 | 90,000 | 0 | 6 |
| 15 | Apr. 4 | Martic Forge.. | Downy woodpecker. | 12 | (a) | (a) | (a) | (a) |
| 16 | Apr. 7 | .....do..... | Brown creeper. | 12 | 40,000 | 80,000 | 0 | 5 |
| 17 | ...do.... | .....do..... | Golden-crowned kinglet. | 12 | 32,849 | 85,357 | 6,566 | 5 |
| 18 | Apr. 10 | 9 miles west of Philadelphia. | Sapsucker..... | 12 | 170,000 | 145,000 | 5,000 | 9 |
| 19 | ...do.... | .....do..... | White-breasted nuthatch. | 12 | 96,045 | 33,900 | 5,655 | 4 |
| 20 | ...do.... | 5 miles west of Philadelphia. | Downy woodpecker. | 12 | 130,000 | 130,000 | 0 | 8 |
| 21 | Apr. 15 | Martic Forge.. | .....do..... | 12 | 3,740,000 | 121,387 | 5,780 | 6 |
| 22 | Apr. 17 | .....do..... | Sapsucker..... | 12 | 344,936 | 337,584 | 7,502 | 9 |
| 23 | Apr. 18 | West Chester.. | Brown creeper. | 14 | 102,309 | 304,004 | 254,019 | 6 |
| 24 | Apr. 21 | Martic Forge.. | Black-and-white creeper. | 14 | 14,925,000 | 90,000 | 0 | 4 |
| 25 | Apr. 25 | .....do..... | Downy woodpecker. | 16 | 358,437 | 159,638 | 27,108 | 9 |
| 26 | Apr. 30 | Martic Forge.. | .....do..... | 14 | 64,338 | 657,169 | 59,742 | 8 |
| 27 | ...do.... | .....do..... | .....do..... | 14 | 6,121,951 | 970,731 | 624,341 | 4 |
| 28 | May 2 | West Chester.. | Black-and-white creeper. | 14 | 478,571 | 28,581 | 0 | 1 |
| 29 | ...do.... | .....do..... | Hairy woodpecker. | 14 | (a) | (a) | (a) | (a) |
| 30 | May 5 | Martic Forge.. | Black-and-white creeper. | 16 | (a) | (a) | (a) | (a) |
| 31 | ...do.... | .....do..... | .....do..... | 16 | (a) | (a) | (a) | (a) |
| 32 | ...do.... | .....do..... | .....do..... | 16 | (a) | (a) | (a) | (a) |
| 33 | May 9 | West Chester.. | Downy woodpecker. | 16 | 1,893,854 | 64,245 | 36,312 | 5 |
| 34 | ...do.... | .....do..... | Black-and-white creeper. | 16 | 18,181 | 28,571 | 0 | 5 |
| 35 | ...do.... | .....do..... | Hairy woodpecker. | 20 | 172,413 | 51,724 | 0 | 5 |
| 36 | May 12 | Martic Forge.. | Black-and-white creeper. | 16 | 24,000 | 16,000 | 0 | 4 |

a Discarded; too heavily seeded with bacteria for a reliable test.

TABLE I.—*Results of tests of birds Nos. 1 to 36 as fungus carriers as shown by cultures*—Continued

SUMMARY

| No. | Kind of bird. | Number tested. | Number carrying Endothia parasitica spores. | Maximum number of spores of Endothia parasitica carried by single bird. |
|---|---|---|---|---|
| 1 | Black-and-white creeper | 7 | 0 | 0 |
| 2 | Brown creeper | 2 | 1 | 254,019 |
| 3 | Downy woodpecker | 16 | 13 | 757,074 |
| 4 | Flicker | 1 | 0 | 0 |
| 5 | Golden-crowned kinglet | 1 | 1 | 6,566 |
| 6 | Hairy woodpecker | 3 | 0 | 0 |
| 7 | Junco | 2 | 1 | 10,000 |
| 8 | White-breasted nuthatch | 2 | 1 | 5,655 |
| 9 | Sapsucker | 2 | 2 | 7,502 |
| | Total | 36 | 19 | |

## RELATION OF RAINFALL TO BIRDS AS CARRIERS OF THE CHESTNUT-BLIGHT FUNGUS

The highest positive results were invariably obtained soon after a period of heavy rainfall, generally one extending over several days. (See Table II.) During the time covered by our tests there were four such rains. (See Table III.) Birds shot from two to four days after each period were found to be carrying the highest numbers of spores of *Endothia parasitica*. Some of the birds shot at other times were also found to be carrying spores of the chestnut-blight fungus, but in much smaller numbers. This relation between the high number of spores and the periods of rainfall is explained by the fact that the pycnospores of the chestnut-blight fungus are produced in large numbers during and after rains and are washed down the trunks of the trees (4). This is explained more in detail later.

TABLE II.—*Rainfall related to birds as carriers of the chestnut-blight fungus*

| Rainfall in inches. | | | | No. of bird. | Locality where shot (Pennsylvania). | Date killed. | Number of Endothia parasitica spores. | Number of fungous spores not Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| Date. | West Chester, Pa. | Date. | Martic Forge, Pa. | | | | | |
| 1913. | | 1913. | | | | 1913. | | |
| Feb. 21 | 0.19 | Feb. 22 | 0.09 | 2 | Martic Forge | Feb. 21 | 0 | 156,600 |
| Feb. 22 | .07 | | | | | | | |
| Feb. 27 | .78 | Feb. 27 | .36 | 3 | do | Feb. 28 | 0 | 80,000 |
| | | Mar. 5 | .02 | 4 | do | Feb. 28 | 10,000 | 115,000 |
| Mar. 9 | .03 | | | 5 | do | Mar. 10 | 30,000 | 612,500 |
| Mar. 10 | .53 | Mar. 10 | .55 | 6 | West Chester | Mar. 19 | 73,333 | 100,000 |
| | | Mar. 13 | 1.29 | | | | | |
| Mar. 13 to Mar. 15 | 1.38 | Mar. 15 | .92 | 7 | do | Mar. 19 | 109,022 | 64,626 |
| | | | | 8 | do | Mar. 19 | 92,000 | 236,000 |
| Mar. 21 | 1.64 | Mar. 21 | .62 | | | | | |
| Mar. 26 | 1.18 | Mar. 26 | 3.47 | 9 | Martic Forge | Mar. 24 | 0 | 60,000 |
| Mar. 27 | | | | | | | | |
| | | | | 10 | do | Mar. 29 | 757,074 | 55,090 |
| Mar. 30 | .30 | Mar. 31 | .04 | 11 | do | Mar. 31 | 15,625 | 246,875 |
| Mar. 31 | .20 | | | | | | | |

TABLE II.—*Rainfall related to birds as carriers of the chestnut-blight fungus*—Continued

| Rainfall in inches. | | | | No. of bird. | Locality where shot (Pennsylvania). | Date killed. | Number of Endothia parasitica spores. | Number of fungus spores not Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| Date. | West Chester, Pa. | Date. | Martic Forge, Pa. | | | | | |
| 1913. | | 1913. | | | | 1913. | | |
| ..... | ..... | Apr. 2 | 0.10 | 12 | York Furnace..... | Apr. 2 | 31,111 | 413,333 |
| ..... | ..... | ..... | ..... | 13 | .....do..... | Apr. 2 | 25,000 | 260,000 |
| ..... | ..... | ..... | ..... | 14 | .....do..... | Apr. 2 | 0 | 90,000 |
| Apr. 4 | .56 | Apr. 4 | .23 | 15 | Martic Forge..... | Apr. 4 | 0 | 0 |
| ..... | ..... | ..... | ..... | 16 | .....do..... | Apr. 7 | 0 | 80,000 |
| ..... | ..... | ..... | ..... | 17 | .....do..... | Apr. 7 | 6,566 | 78,691 |
| ..... | ..... | ..... | ..... | 18 | West of Philadelphia..... | Apr. 10 | 5,000 | 140,000 |
| Apr. 10 to Apr. 13 | 2.49 | Apr. 11 | .55 | 19 | .....do..... | Apr. 10 | 5,655 | 28,245 |
| ..... | ..... | Apr. 15 | 1.23 | 20 | .....do..... | Apr. 10 | 0 | 130,000 |
| Apr. 13 to Apr. 16 | 1.57 | Apr. 16 | 1.15 | 21 | Martic Forge..... | Apr. 15 | 5,780 | 115,607 |
| ..... | ..... | ..... | ..... | 22 | .....do..... | Apr. 17 | 7,502 | 330,082 |
| ..... | ..... | ..... | ..... | 23 | West Chester..... | Apr. 18 | 254,019 | 49,985 |
| ..... | ..... | ..... | ..... | 24 | Martic Forge..... | Apr. 21 | 0 | 90,000 |
| ..... | ..... | ..... | ..... | 25 | West Chester..... | Apr. 25 | 27,108 | 132,530 |
| Apr. 28 Apr. 29 | 2.43 | Apr. 28 | 2.33 | 26 | Martic Forge..... | Apr. 30 | 59,742 | 597,427 |
| Apr. 29 | .11 | Apr. 29 | .07 | 27 | .....do..... | Apr. 30 | 624,341 | 346,390 |
| ..... | ..... | ..... | ..... | 28 | West Chester..... | May 2 | 0 | 28,581 |
| ..... | ..... | ..... | ..... | 29 | .....do..... | May 2 | ..... | ..... |
| ..... | ..... | ..... | ..... | 30 | Martic Forge..... | May 5 | ..... | ..... |
| ..... | ..... | ..... | ..... | 31 | .....do..... | May 5 | ..... | ..... |
| ..... | ..... | ..... | ..... | 32 | .....do..... | May 5 | ..... | ..... |
| ..... | ..... | ..... | ..... | 33 | West Chester..... | May 9 | 36,312 | 27,933 |
| ..... | ..... | ..... | ..... | 34 | .....do..... | May 9 | 0 | 28,571 |
| ..... | ..... | ..... | ..... | 35 | .....do..... | May 9 | 0 | 51,724 |
| ..... | ..... | ..... | ..... | 36 | Martic Forge..... | May 12 | 0 | 16,000 |

TABLE III.—*Relation of maximum number of spores of the chestnut-blight fungus carried to periods of maximum rainfall*

| Rainfall in inches. | | | | Date bird was shot. | Locality where bird was shot (Pennsylvania). | Number of spores of Endothia parasitica carried. |
|---|---|---|---|---|---|---|
| Date. | West Chester, Pa. | Date. | Martic Forge, Pa. | | | |
| 1913. | | 1913. | | 1913. | | |
| Mar. 10 | 0.53 | Mar. 10 | 0.55 | ..... | ..... | ..... |
| Mar. 13 to Mar. 15 | 1.38 | Mar. 13 | 1.29 | ..... | ..... | ..... |
| | | Mar. 15 | .92 | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | Mar. 19 | West Chester..... | 73,333 |
| ..... | ..... | ..... | ..... | Mar. 19 | .....do..... | 109,022 |
| ..... | ..... | ..... | ..... | Mar. 19 | .....do..... | 92,000 |
| Mar. 26 to Mar. 27 | 1.18 | Mar. 26 | 3.47 | ..... | ..... | ..... |
| | | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | Mar. 29 | Martic Forge..... | 757,074 |
| Apr. 10 to Apr. 13 | 2.49 | Apr. 11 | .55 | ..... | ..... | ..... |
| | | ..... | ..... | ..... | ..... | ..... |
| Apr. 13 to Apr. 16 | 1.57 | Apr. 15 | 1.23 | ..... | ..... | ..... |
| | | Apr. 16 | 1.15 | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | Apr. 18 | West Chester..... | 254,019 |
| Apr. 27 to Apr. 28 | 2.43 | Apr. 28 | 2.33 | ..... | ..... | ..... |
| | | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | Apr. 30 | Martic Forge..... | 59,742 |
| ..... | ..... | ..... | ..... | Apr. 3 | .....do..... | 624,341 |

## MICROSCOPIC EXAMINATION OF CENTRIFUGED SEDIMENTS

The sediments from those birds yielding positive results were given a thorough microscopic examination, primarily to ascertain whether the birds were carrying pycnospores or ascospores. (See Table IV.) Ascospores were not found to be present in a single instance. However, in sediments from birds yielding high positive results pycnospores could be found very easily. Where the positive results were not so high, pycnospores were located with more difficulty, but could be found in all sediments except those from birds showing by cultures the smallest number of spores of the blight fungus. The results from cultures substantiate the microscopic examinations, since the rate of development of colonies of the chestnut-blight fungus always indicated their origin from pycnospores (3).

TABLE IV.—*Results of microscopic examination of centrifuged sediments of birds Nos. 1 to 36*

| Bird No. | Number of spores of Endothia parasitica carried, as shown by cultures. | Kind of spores shown by microscopic examination. | Bird No. | Number of spores of Endothia parasitica carried, as shown by cultures. | Kind of spores shown by microcopic examination. |
|---|---|---|---|---|---|
| 1.... | 0 | Examination not necessary. | 20.... | 0 | Examination not necessary. |
| 2.... | 0 | Do. | 21.... | 5,780 | No ascospores. |
| 3.... | 0 | Do. | 22.... | 7,502 | Do. |
| 4.... | 10,000 | No ascospores. | 23.... | 254,019 | No ascospores; pycnospores fairly abundant. |
| 5.... | 30,000 | No ascospores; pycnospores present. | 24.... | 0 | Examination not necessary. |
| 6.... | 73,333 | Do. | 25.... | 27,108 | No ascospores; pycnospores present. |
| 7.... | 109,022 | Do. | | | |
| 8.... | 92,000 | Do. | 26.... | 59,742 | Do. |
| 9.... | 0 | Examination not necessary. | 27.... | 624,341 | No ascospores; pycnospores fairly abundant. |
| 10.... | 757,074 | No ascospores; pycnospores abundant. | 28.... | 0 | Examination not necessary. |
| 11.... | 15,625 | No ascospores; pycnospores present. | 29.... | 0 | Do. |
| | | | 30.... | 0 | Do. |
| 12.... | 31,111 | Do. | 31.... | 0 | Do. |
| 13.... | 25,000 | Do. | 32.... | 0 | Do. |
| 14.... | 0 | Examination not necessary. | 33.... | 36,312 | No ascospores; pycnospores present. |
| 15.... | 0 | Do. | | | |
| 16.... | 0 | Do. | 34.... | 0 | Examination not necessary. |
| 17.... | 6,566 | No ascospores. | 35.... | 0 | Do. |
| 18.... | 5,000 | Do. | 36.... | 0 | Do. |
| 19.... | 5,655 | Do. | | | |

During the time covered by our analyses there were five periods during which ascospores were expelled in the field—i. e., on March 20 and 21, 26 and 27, April 4 and 5, 13 to 16, 27 to 29. On these days ascospores were ejected at both Martic Forge and West Chester, but only a very few spores were expelled on March 20 and 21 and on April 4 and 5. If the remaining dates are compared with Table III, it will be noticed that the birds yielding the highest positive results were shot just after the rains which produced copious expulsion of ascospores. It might therefore be expected that birds would be carrying these spores as well as pycnospores, but such does not appear to have been the case. Studies on wind dissemination show that ascospores are carried away by the wind upon being shot out

of the perithecia. Other work (4) has shown that ascospores are not washed down the trunks of trees by the rains. The birds, therefore, have little, if any, opportunity of collecting ascospores, unless they happen to be working on a canker at the time when expulsion is taking place.

That the pycnospores were not obtained directly from spore horns is indicated by the fact that these were very rare during the earlier part of the period covered by the tests. Furthermore, the number of spores carried by a single bird was much smaller than would be expected if individual spore horns had been brushed off, since a medium-sized tendril is known to contain millions of pycnospores. We know that pycnospores are washed down the trunks of trees in large numbers even by the winter and spring rains (4). Work done in this laboratory shows that viable pycnospores can be obtained in abundance from the healthy bark below lesions. From the facts cited we are led to the conclusion that the pycnospores carried by the birds are brushed off from either normal or diseased bark, or from both, in the movements of the birds over these surfaces.

## BIRDS AS CARRIERS OF OTHER FUNGI

Results from cultures show that a few of the birds (Nos. 7, 10, 23, 27, and 33) were carrying a greater number of viable spores of the chestnut-blight fungus than of all other species of fungi combined. The reverse, however, was true of all other birds, most of which were found to be carrying fungous spores in large numbers. (See Table II.) The number of species of fungi other than *Endothia parasitica* represented in the cultures varied from 4 to 14 per bird, with an average of 7. (See Table I.) Those met with most frequently were various species of Penicillium, Cladosporium, and Alternaria. Many of the other fungi, which appeared in smaller numbers, were not identified, on account of their failure to fruit in culture.

The microscopic examination of the centrifuged sediments revealed the fact that more species of fungi were carried than was indicated by the cultures. Spores of different species were distinguished by form, size, septation, and coloration. For example, the cultures from bird No. 7 indicated the presence of only 7 species other than *Endothia parasitica*, while the sediment showed at least 12 different kinds of spores. Again, bird No. 23 gave 6 species in cultures and at least 19 by microscopic examination of the sediment. The types of spores found in the sediments of these two birds are illustrated by figures 1 and 2. The actual number of fungous spores carried was beyond doubt greater in every case than is indicated in Table I. The smaller number of species obtained from the cultures was due to the fact that the medium used was not suitable to the growth of some of the spores, or that they grew so slowly that they were overrun by other more rapid-growing forms before they had become visible.

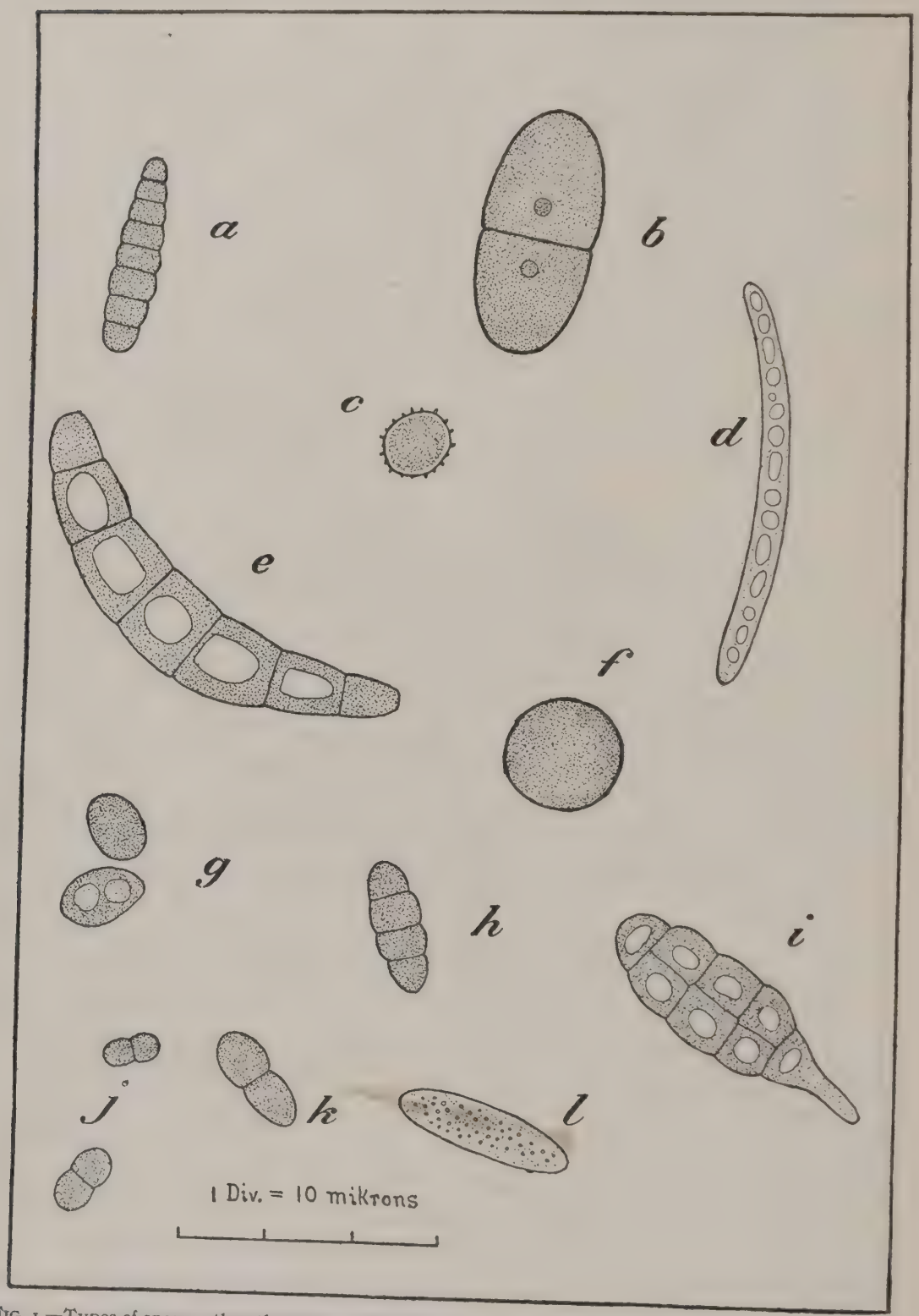



FIG. 1.—Types of spores other than those of *Endothia parasitica* obtained by the microscopic examination of the centrifuged sediment from the test of bird No. 7, a downy woodpecker: *a*, Brown; *b*, dark brown; *c*, brown; *d*, cyanophyceous; *e*, brown; *f*, nearly black; *g*, smoky; *h*, brown; *i*, brown; *j*, brown; *k*, brown; *l*, cyanophyceous.

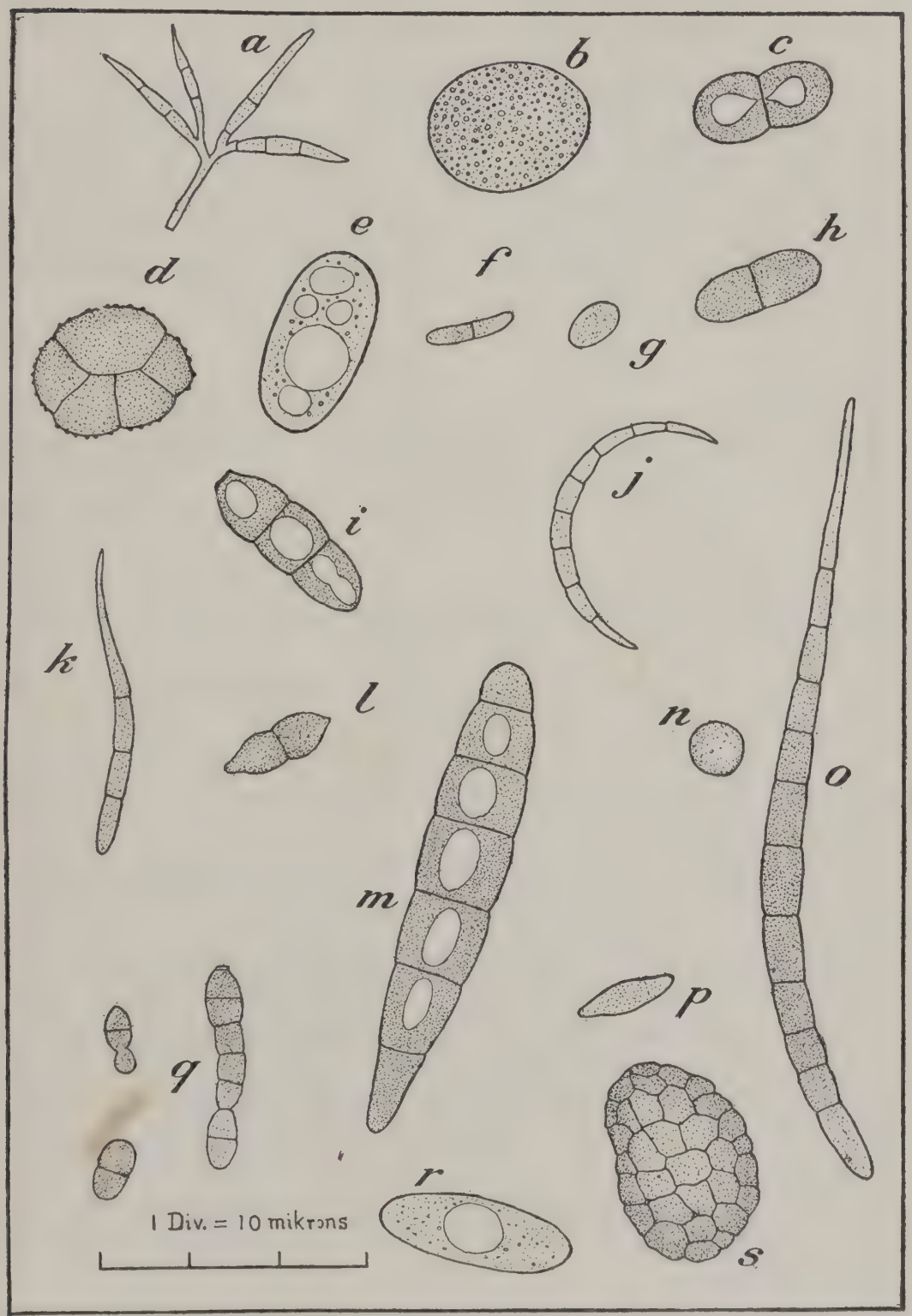



FIG. 2.—Types of spores other than those of *Endothia parasitica* obtained by the microscopic examination of the centrifuged sediment from the test of bird No. 23, a brown creeper. Brown-spored forms appear to predominate: *a*, Hyaline; *b*, hyaline; *c*, dark brown; *d*, nearly black; *e*, hyaline; *f*, light brown; *g*, smoky; *h*, pale smoky; *i*, dark smoky; *j*, hyaline; *k*, pale smoky; *l*, dark smoky; *m*, smoky; *n*, hyaline; *o*, brown; *p*, hyaline; *q*, dark smoky; *r*, hyaline; *s*, very dark, almost black.

No attempt was made to determine whether any of the spores other than those of the chestnut-blight fungus belonged to parasitic species. Judging, however, from the large numbers and kinds of fungous spores carried and from the very high numbers of spores of *Endothia parasitica* obtained at certain times, it is reasonable to suspect that these birds or birds of other species may be important agents in the spread of some other plant diseases, at least under certain favorable conditions. In the light of facts revealed by this investigation it is suggested that birds may play a part in the dissemination of such troubles as the brown-rot of stone fruits, die-back of peaches, plums, and apricots, or of any other diseases where birds may be attracted to the host.

## SUMMARY AND CONCLUSIONS

(1) The 36 birds tested belonged to 9 different species.

(2) Of the 36 birds 32 were those which are in the habit of climbing over the trunk and larger branches of trees.

(3) Most of the birds were shot from blighted chestnut trees; some directly from blight cankers.

(4) The bill, head, feet, tail, and wings of each bird were scrubbed with a brush and poured plates were made from the wash water, which was retained and centrifuged for its sediment.

(5) Of the 36 birds tested, 19 were found to be carrying spores of the chestnut-blight fungus, *Endothia parasitica*.

(6) The viable spores of the chestnut-blight fungus carried by two downy woodpeckers numbered 757,074 and 624,341, respectively, while a brown creeper carried 254,019.

(7) The cultures from some of the birds showed from 2 to 14 times as many viable spores of the chestnut-blight fungus as of all other fungi combined.

(8) The highest positive results were invariably obtained from birds shot from two to four days after a period of considerable rainfall.

(9) The rate of development in cultures always indicated that the colonies of the chestnut-blight fungus originated from pycnospores; pycnospores were generally found in the centrifuged sediments, while ascospores were never detected. The birds were therefore carrying pycnospores only.

(10) The pycnospores carried were probably brushed off from either normal or diseased bark, or from both, in the movements of the birds over these surfaces.

(11) Both the cultures and an examination of the centrifuged sediments showed that the birds were carrying a large number of spores of many species of fungi other than *Endothia parasitica*.

(12) From the above facts the writers are led to the conclusion that birds in general are important carriers of fungous spores, some of which may belong to parasitic species.

(13) Furthermore, many birds which climb or creep over the bark of chestnut trees are important agents in carrying viable pycnospores of the chestnut-blight fungus, especially after a period of considerable rainfall.

(14) Birds are probably not very important agents in spreading the chestnut blight locally, on account of the predominance of other and more important factors of dissemination, as, for example, the wind.

(15) The writers believe, however, that many of the so-called "spot infections" (local centers of infection isolated from the area of general infection) have had their origin from pycnospores carried by migratory birds. Some of the birds tested were not permanent residents of eastern Pennsylvania, but were shot during their migration northward. These, no doubt, carry spores great distances. Each time the bird climbs or creeps over the trunk or limbs of a tree some of the spores may be brushed off and may lodge in crevices or on the rough bark. From this position they may be washed down into wounds by the rain and may thus cause infections.

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PLATE XXXVIII

Old blight canker on chestnut, showing the work of woodpeckers.





## PLATE XXXIX

Series of cultures 9 days old obtained in the test of bird No. 23, a brown creeper. Each Petri dish of the first series (first row) contained 1/10,000 of the water in which the bird was washed; each of the second series (middle row), 1/45,000; and each of the third series (third row), only 1/90,000.

# DENSITY OF WOOD SUBSTANCE AND POROSITY OF WOOD

By Frederick Dunlap,
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## PURPOSE OF THE INVESTIGATION

The investigation the results of which are presented in this paper was made to determine the density of the lignocellulose which makes up the walls of the cells of which wood is composed as a basis for calculating the porosity of wood.[1] The density of a block of wood differs from that of the lignocellulose of which it is built up, because wood is a porous structure and is not a homogeneous solid. Lignocellulose has a density greater than unity and sinks in water; blocks of wood float, especially when dry, because the air they contain buoys them up.

The volume of a block of wood is thus made up of two parts, a framework of lignocellulose and a large number of small cavities inclosed in the former. The cavities may be occupied by a variety of fluids, incrusting materials, and solid substances. The ratio of the sum of the volumes of these cavities to the volume of the entire block is the measure of the porosity of the wood. The volume of the entire block is readily ascertained (by submersion, for example), so that the determination of porosity resolves itself into a determination of the volume of the cavities.

The volume of the cavities may be measured directly by filling them with a fluid of known density; actually such measurements have never met with entire success. The same result can also be reached indirectly by determining the density of the wood substance and then calculating its volume in a block of wood; the difference between the gross volume of the block and the volume of the solid substance is the volume of the cavities.

## RELATION TO TECHNICAL PROBLEMS

If the heat conductivity of wood is greater than that of water, the cavities in wet wood offer an impediment to the flow of heat. If the conductivity of water is greater, the flow of heat is accelerated by its presence in the cavities. Air in the cavities of dry wood will likewise affect heat conductivity. In other words, the conductivity of a block of wood is in part a function of its porosity. The true significance of heat conductivity when measured on blocks of wood can be properly interpreted only when the porosity is known and when the extent to

[1] The research work was carried on at the Forest-Products Laboratory, maintained by the Forest Service in cooperation with the University of Wisconsin, at Madison, Wis.

which observed results depend on wood substance and on air space is calculated. The porosity of wood, then, is an important item in the study of its heat conductivity.

The porosity of wood is also of importance directly in the practice of impregnating wood with preservatives, since the capacity of the cavities fixes an upper limit to the quantity of preservative which may be injected.

## PREVIOUS INVESTIGATIONS

The first published statement of the density of wood substance appears to be that of Hofmeister,[1] who assumed a density of at least 1.45. His assumption was based on measurements of the density of flax fibers, which have a very small lumen. Subsequently other writers assumed a density of 1.55.

In 1879, Sachs [2] published a careful study of the density of wood substance. By washing the air from wood in a current of water and then using Archimedes's method, Sachs obtained a density of 1.5 for *Pinus pumilo* and 1.4 for *Abies pectinata*. Wood boiled in water to expel the air gave somewhat higher densities, and when washed in alcohol the results were still higher—viz, 1.523.

Finally, Sachs suspended thin sections of wood, from 1/10 to 1/5 mm. thick, in solutions of calcium nitrate and zinc nitrate. The density of the solution was adjusted until the sections of wood sank very slowly. It is evident that when the wood remains suspended in the solution the two are of the same density. Sachs read his solution densities from a hydrometer.

Sachs found no difference between determinations made with these two nitrates. He evidently experienced difficulty in reaching exact equilibrium, and was satisfied when his sections sank very slowly through his solutions. He found that sections of *Prunus cerifera* and *Populus dilitata* sank in calcium nitrate solutions of 1.54 density, while *Abies pectinata* sank in a zinc-nitrate solution of 1.56 density. His results were not final, but were merely a somewhat closer approximation than any of his predecessors had secured.

Three years later Hartig [3] determined the density of wood substance for five additional European species—viz, birch, beech, oak, spruce, and Scotch pine. He followed Sachs's method, using calcium nitrate, and apparently met with greater success in establishing equilibrium between his solutions and the wood sections. He found the same value, 1.555, for the density of all five species.

[1] Hofmeister, Wilhelm. Ueber Spannung, Ausflussmenge und Ausflussgeschwindigkeit von Säften lebender Pflanzen. *In* Flora, Jahrg. 45 (n. R. Jahrg. 20), No. 7, p. 105, 1862.

[2] Sachs, Julius. Ueber die Porosität des Holzes. *In* Arb. Bot. Inst. Würsburg, Bd. 2, p. 291–332, 2 fig., 1879.

[3] Hartig, Robert. Ueber die Vertheilung der organischen Substanz, des Wassers und Luftraumes in den Bäumen, und über die Ursache der Wasserbewegung in transpirirenden Pflanzen. 112 p. Berlin, 1882. (Untersuch. Forstbot. Inst. München. Bd. 2.)

## EXPERIMENTAL METHODS AND APPARATUS

The method used in the present research was the same in principle as that followed by Sachs and by Hartig in similar studies on a few European woods 30 years ago. Small blocks were boiled in water until sufficient air was expelled to make them sink. Thin sections were then cut with a sharp knife across the grain. These sections were placed in a solution of calcium nitrate and boiled for a few seconds to complete expulsion of the air; then they were transferred to cold solutions of the same salt. The densities of these cold solutions averaged about 1.6, which was a little greater than the anticipated density of the wood, so that the sections floated on the surface. Water was added, a few drops at a time at intervals of four hours or more, and the solutions held at constant temperature until equilibrium was established and the wood hung suspended in the solution.

A specific-gravity bottle was then submerged in the solution and filled; after a few minutes it was withdrawn, washed, dried, cooled to room temperature, and weighed. The volume of the bottle was determined by filling it with freshly boiled distilled water at the same temperature as the solutions measured, cooling to room temperature, and weighing. These relative densities of the solutions were divided by the density of air-free distilled water at the temperature in question, and a result was obtained expressing the density of the solutions (and also of the wood suspended) in terms of water at its maximum density.

Preliminary experiments showed that accurate results could not be secured without control of the temperature of the solutions. For this purpose a tank 50 cm. on each edge was hung in a wooden box so as to leave a 10-cm. space on four sides and the bottom. This space was filled with sawdust. The tank was open above, but was partially covered with a board when the temperature of the room fell more than 10° C. below the temperature of the water in the tank. A shaft extended through the center of the bottom, and a stirrer made from a 30-cm. electric fan was mounted on this shaft. The stirrer was driven by a ⅛-horsepower electric motor at a speed of 65 revolutions per minute by a belt and through an intermediate shaft for reducing the speed.

The tank was filled nearly full of water. Heat was supplied by a 175-watt carbon filament incandescent lamp submerged in one corner of the tank. The temperature was controlled automatically by an ether thermoregulator—an ether reservoir inverted and sealed with mercury. The recession of the mercury column as the ether contracted broke a dry-cell circuit through a relay and turned on the lamp. A rise in temperature beyond a certain point extinguished the lamp. In this way the temperature of the water in the tank was kept within a range of ½ degree centigrade.

The solutions used were placed in wide-mouthed salt bottles of 150 c. c. capacity and supported in trays, with the water rising to their necks.

## SELECTION AND PREPARATION OF THE MATERIAL

The wood used was taken from material on hand at the Forest-Products Laboratory. The species selected were those studied in investigating the specific heat of wood. The use of the same species in the determination of heat conductivity will result in a series of exactly comparable results.[1]

Table I gives the woods used and the source and nature of each species.

TABLE I.—*Species of woods used in tests, giving nature and source of material*

| Species. | Heartwood or sapwood. | Number of rings per inch. | Locality where grown. |
|---|---|---|---|
| Longleaf pine (*Pinus palustris* Mill.). | Heartwood... | 5 | Tangipahoa Parish, La. |
| Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britt.). | Sapwood..... | 13 | Snoqualmie National Forest, Wash. |
| Pacific yew (*Taxus brevifolia* Nutt.). | Heartwood... | ..... | Columbia National Forest, Wash. |
| Mockernut hickory (*Hicoria alba* (L.) Britt.). | Sapwood..... | 6 | Brandywine, Prince Georges County, Md. |
| Beech (*Fagus atropunicea* (Marsh.) Sudw.). | .....do..... | 3 | Do. |
| Red oak (*Quercus rubra* L.). | Heartwood... | 5 | Richland Parish, La. |
| Sugar maple (*Acer saccharum* Marsh.). | Sapwood..... | 11 | Blue Mountain Forest, Newport, N. H. |

The first determinations of density were made on wood sectioned on a microtome; the wood had been previously prepared by boiling in water and soaking in hydrofluoric acid. Subsequently, to avoid possible errors due to this acid treatment, ½-inch cubes of wood were boiled in water until they sank and were then sectioned with a sharp knife.

The results of 21 determinations are summarized in Table II.

[1] Pacific yew was included in response to an urgent demand for data on this species for use in interpreting results secured in the section of wood preservation in the Forest Products Laboratory.

TABLE II.—*Results of 21 determinations, giving density of various species of wood*

| Species of wood. | Density (referred to water at 4° C.). | | |
|---|---|---|---|
| | Soaked in acid at 35° C. | Soaked in water at— | |
| | | 35° C. | 30° C. |
| Longleaf pine | [a]1.6197±0.0007 | ..... | 1.5060 |
| Douglas fir | 1.5915±0.0012 | 1.5698 | 1.5639 |
| Pacific yew | 1.5534 | ..... | ..... |
| Mockernut hickory | 1.5570 | ..... | 1.5525±0.0003 |
| Beech | 1.5929±0.0005 | ..... | 1.4990±0.0002 |
| Red oak | ..... | ..... | 1.5395±0.0004 |
| Sugar maple | 1.6170 | ..... | 1.5506 |

[a] This is not the "probable error;" the mean values in this column rest on only two observations, and the actual deviation of the two from the mean is indicated.

Table II shows, in the first place, that there are significant differences between the densities of the wood substance from various species of trees. The difference between beech and Douglas fir indicates that the extreme range for these seven species is nearly 4½ per cent.

Those sections which had been soaked in hydrofluoric acid as a step in their preparation for sectioning on the microtome showed a higher density than those which were merely boiled in water. It appears that either some constituent with a density less than 1.55 was removed or some foreign substance with a density greater than 1.55 was added to the wood, or else some molecular rearrangement took place in the wood under the influence of the acid. The chemical analysis which might indicate the actual nature of this change was not undertaken.

The table also gives comparable results of determinations on one species at two temperatures, and from these the thermal expansion of wood substance is shown to be negative. This means that wood substance contracts and becomes denser on heating, instead of expanding and becoming lighter in weight. If this is true, wood substance differs from other substances for which records are available. Moreover, it is difficult to reconcile this anomalous behavior with the well-established fact that, in the aggregate, blocks of wood expand when heated.

The observed increase in density between 30° and 35° C. amounts to one-third of 1 per cent, or one-fifteenth of 1 per cent per degree centigrade. This apparent contraction is about twice as large as the expansion which water undergoes between 30° and 35° C.

## APPLICATION OF RESULTS TO THE CALCULATION OF POROSITY OF CROSSTIES

The following data are excerpted from those given on page 48, in Bulletin 126 of the Forest Service, entitled "Experiments in the Preservative Treatment of Red-Oak and Hard-Maple Crossties," by Francis M. Bond:

*General records on the individual ties*

RUEPING—RED OAK

| Track No. | Oven-dry weight per cubic foot. | Absorption per cubic foot. |
|---|---|---|
| | *Pounds.* | *Pounds.* |
| 2 | 37. 14 | 5. 00 |
| 3 | 36. 93 | 5. 46 |
| 4 | 38. 50 | 4. 24 |
| 5 | 40. 30 | 5. 70 |
| 6 | 40. 70 | 4. 26 |

On the basis of these data the portion of the cell cavity occupied by creosote is readily computed, since it is known that the density of the wood substance of red oak is 1.540; its weight per cubic foot is then 96.06 pounds. The weight of the creosote used in these tests is 65.5 pounds per cubic foot. Table III gives the porosity of the individual crossties computed from the foregoing data.

TABLE III.—*Porosity of red-oak crossties*

| Track No. | Oven-dry weight per cubic foot. | Volume of lignocellulose per cubic foot of wood. | Volume of cavity per cubic foot of wood. | Absorption of creosote. | | Percentage of cell cavity occupied by creosote. |
|---|---|---|---|---|---|---|
| | | | | Pounds per cubic foot of wood. | Cubic feet per cubic foot of wood. | |
| | *Pounds.* | *Cubic foot.* | *Cubic foot.* | | | |
| 2 | 37. 19 | 0. 382 | 0. 618 | 5. 00 | 0. 076 | 12. 3 |
| 3 | 36. 93 | . 384 | . 616 | 5. 46 | . 088 | 14. 3 |
| 4 | 38. 50 | . 401 | . 599 | 4. 24 | . 065 | 10. 8 |
| 5 | 40. 30 | . 420 | . 580 | 5. 70 | . 087 | 15. 0 |
| 6 | 40. 70 | . 424 | . 576 | 4. 26 | . 065 | 11. 3 |

## SUMMARY

To sum up the results of the investigation:

The density of the wood substance in different species of trees may, for practical purposes, be considered as uniform, with a value of 1.54.

Since most commercial woods have a density between 0.3 and 0.6, it is apparent that the unoccupied space in a block of wood may be from four-fifths to two-fifths of its volume.

# PRELIMINARY AND MINOR PAPERS.

## COMPOSITION OF ROQUEFORT-CHEESE FAT

By JAMES N. CURRIE,
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### INTRODUCTION

Many of the problems encountered in attempts to produce a cheese of the Roquefort type in this country can be attributed to differences in composition between sheep's milk and cow's milk. These differ not only in the absolute and relative amounts of fat, casein, milk sugar, and ash, but also in the composition of some of these individual constituents. In a recent publication the author (1914, p. 7) [1] attributed the peppery taste of Roquefort cheese to the accumulation during the ripening process of certain volatile fatty acids of the group insoluble or but partially soluble in water. In view of this it seemed desirable to make a comparative study of the fat of cow's milk and the fat of typical imported Roquefort cheese, with special regard to this group of acids.

Roquefort cheese is made chiefly from sheep's milk. The milk supply of the Roquefort-cheese industry is rigidly inspected by agents of the controlling companies for the express purpose of prohibiting the adulteration of sheep's milk. However, the addition of small amounts of cow's milk and also of goat's milk is admitted by the cheese makers. Marre (1906, p. 53) gives the following statistics concerning the milk used in the manufacture of Roquefort cheese for 1904:

TABLE I.—*Statistics of the manufacture of Roquefort cheese in 1904*

| Kind of animal. | Number of animals. | Milk produced. | Proportion of milk. |
|---|---|---|---|
| | | *Hectoliters.* | *Per cent.* |
| Ewes | 521,330 | 329,420 | 97.36 |
| Cows | 1,569 | 8,337 | 2.46 |
| Goats | 699 | 605 | .18 |

For the purpose of this investigation it was thought that a study of the undecomposed fat of typical imported Roquefort cheese would be fully as significant as a study of the fat of sheep's milk, although it is recognized that the two may not be strictly identical.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 421.

Milk fat is such a complex substance that the exact differences in composition between the milk fats of the various mammals are difficult to determine. That there are marked differences has been shown by Pizzi (1894), who determined the Reichert-Meissl number of the milk fat of 12 mammals. His results are summarized as follows:

TABLE II.—*Reichert-Meissl number of the milk fat of 12 mammals, according to Pizzi*

| Mammal. | Reichert-Meissl number. | Mammal. | Reichert-Meissl number. | Mammal. | Reichert-Meissl number. |
|---|---|---|---|---|---|
| Sheep | 32.89 | Rabbit | 16.06 | Rat | 2.97 |
| Goat | 28.60 | Ass | 13.09 | Sow | 1.65 |
| Cow | 27.00 | Mare | 11.22 | Woman | 1.42 |
| Buffalo | 26.18 | Cat | 4.40 | Dog | 1.21 |

Such differences in the volatile-soluble acids as are shown in the above results suggest differences in the relative proportions of the other acids present.

A thorough search of the literature of the subject has not revealed any report of a complete analysis of sheep's-milk fat. Fodor (1912) determined some of the chemical constants through an entire period of lactation. He obtained the fat by separating it from fresh Liptauer cheese made from sheep's milk. Martin (1914) examined the fat of milk known to be genuine sheep's milk from the locality of Roquefort at weekly intervals from January 28 to June 2. The results of these two investigators, together with the results of Eckles and Shaw (1913) for two breeds of dairy cows, are summarized in Table III.

TABLE III.—*Chemical constants of the fat of sheep's and cow's milk*

| Investigator. | Source of milk fat. | Reichert-Meissl number. | Iodin number. | Saponification number. | Polenske number. | Caprylic-acid number (Dons). |
|---|---|---|---|---|---|---|
| Fodor | Liptauer cheese | 26.84 | 39.3 | 231.5 | ..... | 2.9 |
| Martin | Sheep's milk | 28.48 | ..... | 231.58 | 4.40 | ..... |
| Eckles and Shaw. | Jersey milk | 26.73 | 30.52 | 228.9 | ..... | ..... |
| Do | Holstein milk | 26.28 | 34.20 | 229.1 | ..... | ..... |

The Polenske and caprylic-acid numbers are figures based upon arbitrary procedures and are proportional to the volatile-insoluble acids. Polenske states that his number for a cow's-milk fat having a Reichert-Meissl number of 26 to 27 is 1.9 to 2. Dons gives the caprylic-acid number for cow's-milk fat as 1.75.

From these chemical constants it appears that the quantity of volatile-insoluble acids of sheep's-milk fat is about double that of cow's-milk fat. The oleic-acid content of the former is also greater than that of the latter.

## VOLATILE ACIDS OF ROQUEFORT-CHEESE AND COW'S-MILK FAT

Four Roquefort cheeses of different brands and ripened as little as any to be found in the market were procured and the fat separated by the Schmidt-Bondzynski method. In order to remove any free fatty acids,

the fat was shaken out three times in a separatory funnel with twice its volume of 95 per cent alcohol warm enough to keep the fat in a fluid state. There are certain objections to treating a fat with alcohol (Lewkowitsch, 1909), but this seems to be the only practicable way of removing free acids insoluble in water. The fat was then washed three times with hot water to remove the alcohol, dried, and filtered clear. Fat treated in this way had a decinormal acid number for 10 grams of about 3.0, which is very nearly the same as the acid number of fresh, filtered fat of cow's milk.

The Reichert-Meissl and the Polenske numbers were determined on these four samples of fat and also on a sample of cow's-milk fat, with the following results:

TABLE IV.—*Reichert-Meissl and Polenske numbers of the fats of Roquefort cheese and cow's milk*

| Source of fat. | Reichert-Meissl number. | Polenske number. |
|---|---|---|
| Roquefort cheese: | | |
| Elite | 29. 62 | 5. 55 |
| Roquefort Belier | 26. 72 | 6. 25 |
| Mialane et Cie | 26. 35 | 5. 68 |
| Louis Rigal | 25. 64 | 5. 60 |
| Cow's-milk fat | 27. 27 | 2. 00 |

By direct distillation, as in the Reichert-Meissl number determination, only a part of the volatile acids are removed. The total quantity can be more nearly estimated by distillation with steam. Five-gram samples of two of the cheese fats and of the cow's-milk fat were saponified and distilled with steam to 1,000 c. c. The titers of the soluble and insoluble acids are given in Table V.

TABLE V.—*Quantity of soluble and insoluble volatile acids in 5 grams of Roquefort-cheese fat and of cow's-milk fat determined by distillation with steam*

[Expressed in c. c. of N/10 acid.]

| Source of fat. | Soluble acids. | Insoluble acids. |
|---|---|---|
| Roquefort cheese (Elite) | 41. 00 | 18. 01 |
| Roquefort cheese (Mialane et Cie.) | 38. 80 | 19. 20 |
| Cow's-milk fat | 36. 00 | 11. 17 |

The physical properties and also the molecular weights of the insoluble acids showed that an acid of greater molecular weight than capric distilled over with the steam. In order to determine more accurately the quantity of each acid present, the distillate was divided into four fractions. The volatile acids of the first 500 c. c. of distillate constituted fraction 1, and the insoluble acids draining out of the condenser with this 500 c. c. of distillate formed fraction 2. Fraction 3 consisted of the soluble acids of the second 500 c. c. of distillate, and the remainder of the insoluble acids constituted fraction 4. The composition of fraction 1 was determined by a Duclaux distillation, and the compositions of fractions 2, 3, and 4

were determined from the weights of the barium salts and the resulting barium sulphate on moistening with dilute sulphuric acid and igniting. The quantity of each acid in fractions 2, 3, and 4 was estimated by assuming that each consisted of the two acids between which the mean molecular weight of the fraction fell. Only traces of soluble acids distil after the first 1,000 c. c., and the insoluble acids distilling between 1,000 and 1,500 c. c. have a molecular weight greater than that of lauric acid. Hence, it is believed that the first 1,000 c. c. of distillate contains practically all the acids from butyric to capric, inclusive.

By the above process of fractionation the volatile acids of one of the cheese fats (Elite) and of the cow's-milk fat have been estimated. The results are shown in Table VI.

TABLE VI.—*Volatile acids of Roquefort-cheese fat and of cow's-milk fat*

ROQUEFORT-CHEESE FAT

| Fraction No. | $BaSO_4$ from barium salt. | Lauric acid. | | Capric acid. | | Caprylic acid. | | Caproic acid. | | Butyric acid. | | Total acid. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P. ct.* | *C. c.*[a] | *Gms.* | *C. c.*[a] | *Gms.* | *C. c.*[a] | *Gms.* | *C. c.*[a] | *Gms.* | *C. c.*[a] | *Gms.* | *C. c.*[a] |
| 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 19.02 | 0.2206 | 19.79 | 0.1742 | 38.81 |
| 2 | 47.47 | 1.89 | 0.0378 | 8.63 | 0.1485 | ..... | ..... | ..... | ..... | ..... | ..... | 10.52 |
| 3 | 58.44 | ..... | ..... | ..... | ..... | 2.01 | 0.0289 | 1.37 | .0157 | ..... | ..... | 3.38 |
| 4 | 44.86 | 7.27 | .1454 | 2.43 | .0417 | ..... | ..... | ..... | ..... | ..... | ..... | 9.70 |
| Total | 150.77 | 9.16 | .1832 | 11.06 | .1902 | 2.01 | .0289 | 20.39 | .2363 | 19.79 | .1742 | 62.41 |
| Per cent. | ..... | ..... | ..... | ..... | 3.80 | ..... | .58 | ..... | 4.73 | ..... | 3.48 | ..... |

COW'S-MILK FAT

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ..... | ..... | ..... | ..... | ..... | 3.73 | .0537 | 11.20 | .1299 | 22.57 | .2023 | 37.90 |
| 2 | 46.55 | 1.20 | .0240 | 1.70 | .0292 | ..... | ..... | ..... | ..... | ..... | ..... | 2.90 |
| 3 | 55.08 | ..... | ..... | ..... | ..... | 1.63 | .0235 | ..... | ..... | ..... | ..... | 1.63 |
| 4 | 42.53 | 7.69 | .1538 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7.69 |
| Total | ..... | 8.89 | .1778 | 1.70 | .0292 | 5.36 | .0772 | 11.20 | .1299 | 22.57 | .2023 | 50.12 |
| Per cent. | ..... | ..... | ..... | ..... | .58 | ..... | 1.54 | ..... | 2.60 | ..... | 4.05 | ..... |

[a] Acidity expressed in c. c. of N/10 acid.

The butyric acid was also estimated in another of the cheese fats (Mialane et Cie). It contained only 2.99 per cent, which is even less than was shown by the cheese in Table VI. These data show that capric and caproic acids are present in much greater quantities in sheep's-milk fat than in cow's-milk fat.

## SEPARATION OF NONVOLATILE ACIDS OF ROQUEFORT-CHEESE FAT

A fractional separation of the nonvolatile acids of milk fat presents many more difficulties than a separation of the volatile acids. Such a separation involves numerous approximations, but inasmuch as it was deemed desirable to extend the comparative study of the cheese fat and cow's-milk fat to the insoluble-acid portion, the method of Browne (1899) was applied to purified Roquefort-cheese fat.

A sample of 90.29 grams of fat was saponified, washed with 10 liters of water, and separated into seven fractions. The method of Browne was followed, except in some details. Fraction 6 was obtained by

diluting the ammoniacal solution with water as long as a precipitate separated, and fraction 7 by evaporating the entire filtrate from 6, acidifying with sulphuric acid, and extracting with ether. The 90.29 grams of fat yielded 80.8707 grams of insoluble acids, of which 73.5933 were recovered in the seven fractions.

In this process of fractionation the disturbing factor is oleic acid, which not only contaminates every fraction, but also further complicates the problem by giving rise to considerable quantities of dioxystearic acid during the manipulation. From the quantity of oleic acid recovered, which was calculated from the iodin absorption numbers of the several fractions, it was estimated that 23 per cent of the oleic acid was converted to dioxystearic acid. There is a possibility that other unsaturated acids, more readily oxidized than oleic acid, were present, but this problem has not been investigated.

With the foregoing assumptions the composition of the seven fractions was calculated. The results are shown in Table VII.

TABLE VII.—*Weight in grams of the insoluble acids recovered by fractionating 80.8707 grams of insoluble acids of Roquefort-cheese fat*

| Acid. | Fraction No. | | | | | | | Total quantity of acid. |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Oleic | 4.6893 | 2.2424 | 5.9223 | 0.9262 | 1.3358 | 10.7260 | 1.1558 | 26.9978 |
| Dioxystearic | 1.2101 | .5786 | 1.5281 | .2390 | .3447 | 2.7676 | .2982 | 6.9663 |
| Stearic | 1.7278 | ..... | ..... | ..... | ..... | ..... | ..... | 1.7278 |
| Palmitic | 17.9023 | 3.2793 | 4.4381 | .1412 | ..... | ..... | ..... | 25.7609 |
| Myristic | ..... | 2.0347 | 5.1170 | 1.4832 | 1.6289 | ..... | ..... | 10.2638 |
| Lauric | ..... | ..... | ..... | ..... | .0527 | .6702 | ..... | .7229 |
| Capric | ..... | ..... | ..... | ..... | ..... | .2265 | .9273 | 1.1533 |
| Total | 25.5295 | 8.1350 | 17.0055 | 2.7896 | 3.3621 | 14.3903 | 2.3813 | 73.5933 |

If a correction be made for the oxygen absorbed, the acids not recovered amounted to 7.4045 grams. This loss would obviously consist chiefly of oleic, lauric, and capric acids. The amount of oleic acid was known from the iodin number of the fat, and the capric acid was assumed to be 3.80 per cent of the original fat. The lauric acid could therefore be obtained by difference. The final results, together with the results given by Browne for cow's butter, are summarized in Table VIII.

TABLE VIII.—*Percentage of acids in the fats of Roquefort cheese and cow's milk*

| Acid. | Roquefort cheese. | Cow's milk (Browne). | Acid. | Roquefort cheese. | Cow's milk (Browne). |
|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | | *Per cent.* | *Per cent.* |
| Butyric | 3.48 | 5.45 | Myristic | 11.36 | 9.89 |
| Caproic | 4.73 | 2.09 | Palmitic | 28.53 | 38.61 |
| Caprylic | .58 | .49 | Stearic | 1.91 | 1.83 |
| Capric | 3.80 | .32 | Oleic | 38.10 | 32.50 |
| Lauric | 5.84 | 2.57 | Dioxystearic | ..... | 1.00 |

## CONCLUSIONS

The differences between the fat of typical imported Roquefort cheese and the fat of cow's milk are not great enough to warrant the exclusive use of sheep's milk in the manufacture of this type of cheese. However, it is evident that an imported cheese, made wholly or chiefly from sheep's milk, will have more of the peppery taste than a cheese of the same ripeness made from cow's milk.

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# A NEW SARCOPHAGID PARASITE OF GRASSHOPPERS[1]

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## INTRODUCTION

On a bright, sunny afternoon early in September, 1908, near Wellington, Kans., the writer noted hundreds of grasshoppers (*Melanoplus differentialis* Thos. and *M. bivittatus* Say) upon tall weeds which grew in abundance along the sides of the road. They were continually flying in front of him, when suddenly his attention was attracted to flies which were striking the grasshoppers, causing the latter to drop to the ground as if shot. Several of the fallen grasshoppers were examined, but no eggs could be found on them, and no attempt was made at that time to rear possible parasites.

On July 31, 1912, the writer caught an individual of *Melanoplus differentialis* as it was being struck by flies, both flies and grasshopper being captured, but, as before, a close examination of the grasshopper indicated that no eggs had been deposited upon it.

The flies, which at first were thought by the writer to be tachinids, were preserved, and the grasshopper, together with four others that had been struck by flies, was caged in an effort to rear the parasites. The grasshoppers died on August 4 and 6. Full-grown maggots issued from these dead grasshoppers on August 10, pupated from August 12 to 14, and adults, identical with those collected on July 31, issued on August 26. These flies were determined by Mr. W. R. Walton, of the Bureau of Entomology, as belonging to several species, one of which has since been determined as undescribed by Dr. J. M. Aldrich. (See description on p. 443–444 as *Sarcophaga kellyi*.) These rearings seemed to furnish ample proof that the species under observation were parasitic, and the writer supposed that in striking the grasshoppers the flies were ovipositing.

The peculiar, quite shrill tone produced by the flying sarcophagids is very characteristic, and being so much louder than that of other flies, it greatly facilitates observations.

## FIELD STUDIES OF SARCOPHAGIDS

On September 17, 1912, a number of grasshoppers, which had been struck by the fly described herein as *Sarcophaga kellyi* Aldrich, were caught and examined closely, but again no eggs could be found on them. A grasshopper upon which no eggs were to be found was then caught and released in the vicinity of five of these flies, all of which were sitting on a block of wood. As soon as the grasshopper was released, the flies darted after and struck it, causing it at once to drop to the ground. It was caught again for examination, but, as before, no eggs could be found upon it. This experiment was repeated several times, always with the same negative results. After this continued fruitless searching for eggs, a number of

[1] Description of new species by Dr. J. M. Aldrich, Entomological Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology.

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the grasshoppers were collected for the purposes of rearing and dissection. Dissection revealed several small maggots in the viscera of the thorax and abdomen and tiny maggots under the scutellum of the metathorax. Flies were then captured and preserved for reference.

On September 19 further investigations of the supposed oviposition of this sarcophagid were undertaken in a different manner. A female fly was caught, and upon dissection the uterus was found to be full of tiny larvæ, indicating that the flies were viviparous. A search was then made on the bodies of grasshoppers for the tiny maggots, and they were readily found on the metathorax, just beneath the scutellum. More critical examinations and observations showed that tiny larvæ were placed by the fly on the underside of the unfolded posterior wings of the flying grasshopper, the striking of the wing by the fly probably causing the sudden dropping of the victim.

A large grasshopper (*Schistocerca americana* Drury) was captured, and when it attempted to fly, while being held by its hind legs, it was at once struck on the underside of the unfolded wing by several sarcophagids. By repeating this experiment in inducing it to attempt flight, the writer was thus afforded opportunity to observe more carefully the larviposition habit of the fly. Several individuals of *Melanoplus atlanis* Riley and *M. femur-rubrum* DeGeer were also collected and the undersides of their wings examined to determine whether this habit of placing the maggots on the underside was usual. On no less than 32 specimens the maggots were found on the underside of the unfolded wing, with a few also placed promiscuously on the abdominal segments. An examination of 75 individuals of *M. bivittatus* gave similar results.

An examination of the folded wing of a grasshopper which had been struck by a fly and suddenly dropped to the ground disclosed the tiny maggots crawling toward the base of the wing, using the sides of the fold for a trough in which to travel. Thus they reach the base of the wing and the metathorax, where the body is quite soft and moist, and enter the body of their victim to feed upon the internal vital organs. They grow rapidly, maturing in from 10 to 30 days. Not all of the maggots, however, are placed on the wing; some are deposited on segments of the abdomen, and these enter through the segmental divisions. After becoming full grown, the larvæ crawl from the body of the grasshopper and enter the soil to a depth of from 2 to 6 inches, pupate, and later emerge as adult flies.

While they are most frequently found beneath the host, the larvæ do not always enter the soil immediately beneath the dead grasshopper, but have sometimes been observed to crawl a distance of 40 inches before descending into the ground.

The sarcophagid larvæ removed from the uterus of the female, as well as those deposited on the grasshoppers, are quite small, tapering from the thoracic segments almost to a point at the posterior extremity. The segments are bordered by a heavy fringe of short, dark bristles or hairs that give the larvæ a banded appearance. The head tapers slightly and is fitted with a pair of toothed hooks that project laterally. The hooks and bristles are used by the maggot in clinging to and working its way into the body of the host. The maggots, soon after entering the host, lose the dark-colored bristles, becoming of a creamy white color and retaining this color until their emergence from the grasshoppers to enter the soil.

A peculiarity of the sarcophagid flies is that they are often unable to distinguish grasshoppers from other insects; they were observed to strike moths and butterflies, actually depositing larvæ upon them. However, attempts to rear the flies from moths and butterflies so attacked were unsuccessful.

While in the midst of these observations a cicada (*Cicada tibicen* L.) flew up, and no less than a dozen flies flew after it. Owing to the exceedingly strong flight of the cicada it could not be captured. The species attacking the cicada could not be determined, but apparently it was *Sarcophaga kellyi*, as this species was the only one collected in the field at that time. The following note by Mr. Theodore Pergande, now in the files of the Bureau of Entomology, indicates, without proving it, that sarcophagids can develop in *C. tibicen*.

On August 22, 1894, Mr. Thomas J. Brady, of Colonial Beach, Va., sent one dead specimen of *Cicada tibicen* to Washington; from it emerged a number of dipterous larvæ, the adults of which have been determined by Dr. Aldrich as *Sarcophaga helicis*.

As further illustrating the indiscriminate deposition by the flies, the writer crumpled a piece of tissue paper and threw it into the wind among them, when no less than half a dozen flies struck it. When the paper was examined, two tiny maggots were found clinging to it.

In July, 1913, nymphs of *Melanoplus differentialis* and *M. bivittatus* became quite plentiful, affording ample material for study. Adults of *Sarcophaga kellyi* were very common, depositing on nymphs of grasshoppers in the second, third, and fourth instars as they hopped about, but in no instance could a fly be observed depositing on those not in motion.

Mr. H. E. Smith, of the Bureau of Entomology, repeatedly observed flies depositing on nymphs of *Dissosteira longipennis* Thos., on June 16, 1913, during a severe outbreak of this species at Elida, N. Mex. His observations are as follows:

Immediately and for some time after molting, the grasshopper is very soft and by no means active, but crawls upon some vegetation in order to dry. The female sarcophagid flies in amongst weeds, etc., where the grasshoppers are drying themselves, crawls upon them, and though the grasshoppers kick, but not vigorously, sticks living maggots beneath the posterior end of the thoracic sculpture.

Mr. Smith succeeded in collecting enough of the depositing flies to substantiate his observations on the methods of larviposition; and, besides, a large number of *Sarcophaga kellyi* were reared at the Wellington, Kans., laboratory from material collected by him at Elida. Possibly this species in common with others utilizes more than one method of larviposition, thus responding to a variety of stimuli in depositing their young.

Quite a serious outbreak of grasshoppers occurred in the vicinity of Wellston, Okla., early in June, 1913, the prevalent species being *Melanoplus differentialis*, *M. bivittatus*, and *M. atlanis*, with a few scattering individuals of other species, both imagoes and nymphs doing much damage to corn and alfalfa and literally swarming in grasslands. The ground was strewn with dead nymphs and adults of the three species mentioned which had died from parasitism by sarcophagids, their bodies being alive with maggots, while the fields were also literally swarming with these flies engaged in striking adults and nymphs of each instar, except the first, but deposition took place only while grasshoppers were flying, or, in the case of the nymphs, hopping. The winged grasshoppers

appeared to know that the parasites were after them, as when they took wing they made many twists and turns in attempting to get away from the flies. Several adults of *Sarcophaga kellyi* were reared from this Wellston material, while later investigations indicated that the grasshoppers had been materially reduced and practically controlled, so that in late September few eggs were to be found.

## REARING EXPERIMENTS WITH SARCOPHAGIDS

In order to get ample material for study and identification of the species involved, a lot of parasitized grasshoppers were collected and placed in rearing cages. Seventy-two grasshoppers containing a number of these sarcophagid larvæ were collected at the edge of a wheat field on October 21, 1912, and were put in a closed receptacle containing soil; on November 6 following, 97 sarcophagid larvæ were removed from the soil. On December 3, 24 of these pupated and were removed to a receptacle especially designed for rearing Diptera. The remaining 73 larvæ were allowed to continue for hibernation in the soil, which was placed in flowerpots in the laboratory, where they began to pupate on February 3, 1913, adults issuing from the former lot of 24 by the middle of February and from the latter 73 in early March.

On November 8, 1912, 118 living grasshoppers were collected in the same wheat field from which the 72 dead individuals previously mentioned were obtained. Owing to the lateness of the season, these grasshoppers were quite sluggish, but dissection of some of them revealed nearly grown Sarcophaga maggots, as many as 5 to 9 larvæ being removed from some of the grasshoppers. The remaining living grasshoppers were placed in a Riley rearing cage outdoors near the laboratory, but all of them were dead by November 28. The soil of this cage was examined on December 5, and 137 sarcophagid larvæ were removed from it, 75 of these being placed in a large flowerpot and removed to the laboratory, while the remainder were placed in a similar flowerpot which was buried in the soil in the field. These two cages were covered with wire screens. From the indoor cage adults began to issue on February 24, 1913, and continued to issue until May 3. From the flowerpot placed in the field adults began to issue on March 8, continuing to issue until May 5, when the soil was examined and the puparia were all found to be empty.

From the bodies of some 800 dead grasshoppers collected during the fall of 1912 nearly 1,200 Sarcophaga of several species issued. About 800 of these were kept inside in flowerpots and other rearing receptacles, from which adults began to issue about the middle of February, continuing to issue until early May. About 400 of this lot of larvæ were placed in large flowerpots, securely covered, and buried in the soil in the field. An examination of one of these flowerpots in mid-December while the ground was thoroughly frozen indicated that the Sarcophaga were hibernating as larvæ. These larvæ were at once returned to the soil, but unfortunately they were killed by the transfer. Another of these outdoor flowerpots was examined in February. It being then clear that the larvæ had not yet pupated, continued examination until March 8 was made necessary in order that the date of pupation under natural conditions might be learned.

Adults from the flowerpots in the field began to issue in late March and continued emerging until May 28. Comparison of the adults reared

from the Sarcophaga larvæ that issued from grasshoppers collected after death with the adults of Sarcophaga issuing from the living grasshoppers which died in confinement revealed the fact that they were the same species. The indoor and outdoor rearings were indicative of their natural hibernating habits, and subsequent rearings from larvæ from the fields in the spring gave proof of the habit.

## SEASONAL HISTORY AND NUMBER OF GENERATIONS

Observations on the habits of species of Sarcophaga in their relation to grasshoppers were continued in 1913 in the vicinity of Wellington, Kans., beginning with the issuance of the first adults in late April and early May, which was simultaneous with the entering of the second and third instars by the earliest hatched *Melanoplus differentialis* and *M. bivittatus*. At the same time *Chortophaga viridifasciata* DeGeer, a species of grasshopper that hibernates in the nymphal stages, was becoming plentiful in both the adult and nearly grown nymphal forms. The four species, *Sarcophaga kellyi*, *S. cimbicis* Towns., *S. hunteri* Hough, and *S. sarraceniae* Riley, were observed depositing on adults and nymphs of the last species and on the larger nymphs of the other two species, which, however, were not very common. Adults of these four species, which constituted a distinct second generation, began issuing sparingly from these grasshoppers by the first week of June and did not become very much in evidence until about the first week of July, and from this time until November no distinction could be made between generations on account of overlapping. However, judging from the rapidity of their development, there were probably three or four additional generations. making about five or six for the season.

## EFFECT OF POISON ON SARCOPHAGID MAGGOTS

About 200 living grasshoppers that had eaten poisoned bran were collected on October 3, 1913, and by the 17th all had died. Of these grasshoppers 117 contained dead Sarcophaga larvæ and no live ones, as many as 9 larvæ being found in 1 individual. These maggots, with the exception of one which was nearly full grown, were rather small. The use of the poisoned bran in this instance was very effective, for in addition to clearing the field of the grasshoppers it killed the parasites. Poisoned bran was also very effective in the vicinity of Kinsley, Kans.

## OUTBREAKS OF GRASSHOPPERS REDUCED OR CONTROLLED BY SARCOPHAGIDS

The outbreak of *Dissosteira longipennis* in eastern New Mexico, previously mentioned, was considerably reduced by the attack of Sarcophaga larvæ, according to notes made by Mr. H. E. Smith on June 24, 1913, in which he says:

> Found thousands of grasshoppers that had been killed by Sarcophaga larvæ lying dead on the prairie. In some places as many as 15 per square foot were found in this condition.
>
> While in the majority of cases the maggots were still feeding within the bodies of their victims, many full-grown maggots had issued and could be found buried ½ to 2 inches below the surface of the soil.

The investigations by Mr. Smith ended about the 1st of July, and no data as to the results of this parasitism were collected later. Mr. F. R.

Meadows, of American Falls, Idaho, reported on July 22, 1910, that a fly had destroyed the grasshoppers which had been so destructive in that locality during the three preceding years. Unfortunately, specimens of the flies submitted with the letter can not now be located.

Mr. C. B. Neihart, Coulee, Wash., in a letter to Mr. George I. Reeves, of the Bureau of Entomology, dated October 9, 1907, makes the following statement:

> Crops badly injured by grasshoppers; latter now dying by millions. Inclosed specimens collected, some separately, others in bodies of grasshoppers out of which they came after hoppers were put in alcohol. Farmers say that 10 days ago they had no hope of getting crops, but now prospect is good.

Mr. Reeves observed many specimens of *Melanoplus biliturus* Walk. and especially of sarcophagid flies in the fields in a draw at Wilson Creek, Wash., on June 22, 1908. He collected 10 flies, 8 of them having been determined by Dr. Aldrich as *Sarcophaga kellyi* and the other 2 as *S. hunteri*. Mr. Reeves stated that he casually observed one female ovipositing on a Melanoplus, but he unfortunately did not describe the method of the oviposition.

Mr. C. N. Ainslie observed grasshoppers swarming in alfalfa fields in the vicinity of Payson, Utah, in July, 1911. The following note was made by him at that time and is given in full because the flies that he observed have been identified by Dr. Aldrich as *Sarcophaga kellyi* and partially confirm the writer's observations:

> July 16, 1911. While in the vicinity of Payson, 65 miles south of Salt Lake, grasshoppers, mostly immature, were observed swarming in most of the alfalfa fields of that vicinity. Several species appeared to be represented.
>
> In connection with this grasshopper infestation, swarms of flies, large fellows with tessellated abdomens, were everywhere in evidence, being particularly numerous in the vacant spaces in the field that had previously been devastated by the ant, Pogonomyrmex. The numbers of these flies were so great that the hum of their flight was almost equal to that from a swarm of bees. Ten days later in the same locality the grasshoppers were found to be still extremely numerous, both in the stubble and in the alfalfa fields. Swarms of the same species of flies were also observed as on a previous visit. Most of the dead grasshoppers were to be found beneath the alfalfa stems, their bodies being now mere shells, dry and brittle, crushing with the least touch. On digging in the earth beneath some of these dead bodies a number of dipterous puparia were found at depths varying from ½ to 2 inches. The dipterous larvæ, before transforming, seemed to seek damp ground, and in some cases did not descend vertically from the host. In one case a fresh puparium was taken directly from the body of the host, but that was apparently not a common thing.
>
> In one case four large dipterous larvæ were removed from the body of a single grasshopper, two being present in several cases. One crippled grasshopper that could still jump feebly was examined and a small larva was found in its abdomen.
>
> The flies were present in enormous numbers, and as one walked through the alfalfa they would rise in flight, seeming to have unbounded curiosity, and at different times while I was lying on the ground taking notes in the field they would alight on me in large numbers. In several instances as many as from 40 to 50 were counted on my arms and shoulders, resting in the sun. On the wing they are as quick as a flash, as was shown by the manner in which they pursued the winged grasshoppers.
>
> In a stubble field adjoining the alfalfa in which I was making my observations were quite a number of the large-winged grasshoppers (Hippiscus ?) that would take wing as I neared them. In almost every case when one would rise to fly it was pursued by a small swarm of flies; in some cases by as many as a dozen, although the flight was so rapid that nothing more than a guess was possible as to the number of the pursuing flies. The chase was not a mere chance flight, for the flies would mass about the flying grasshopper just as angry bees will gather about one's head in case of pursuit. Dragonflies (Odonata) were not molested in this way, and there seemed to be something in the flight of the grasshoppers that invited pursuit.
>
> August 25, 1911. Studied to-day the grasshopper situation in the same neighborhood as before. Less grasshoppers were present than on July 26. The leafless stems

in the particular field studied had been cut and a new growth had come up that showed little damage. Plenty of dead grasshoppers still lay on the ground beneath the alfalfa, but I could find none that appeared to have recently died. The swarms of flies present in July had diminished somewhat, but many were still on hand. Counted 18 on one arm and shoulder during a brief halt in the field to-day, and noticed repeatedly the same instantaneous dash of these flies for the flying grasshoppers that had been observed before.

It would seem that these parasitic flies have reduced very materially the number of the grasshoppers in this region, but further observation is needed to confirm this—another year.

## SPECIES OF SARCOPHAGA KNOWN TO PARASITIZE GRASSHOPPERS

Several other less abundant species of Sarcophaga were observed in the act of larvipositing on grasshoppers and were subsequently reared from them during these investigations—notably, *Sarcophaga cimbicis*, *S. sarraceniae*, and *S. hunteri*, and in addition to these *S. helicis* Towns. was reared from the dead grasshoppers.

While there are on record many observations strongly indicating that several species of Sarcophaga may be parasitic upon grasshoppers, unfortunately absolute proof as to whether they actually are parasitic upon living grasshoppers or are scavengers feeding upon the dead bodies of grasshoppers has heretofore been wanting. Some of these records are incomplete, through no fault of the observer. As an illustration, Dr. Aldrich stated to the author that he had reared a great number of sarcophagids from grasshoppers at Market Lake, Idaho, during an outbreak in 1898, but unfortunately this material was destroyed by a fire in the University of Idaho, so that no determinations of the material could be made.

Dr. Aldrich has, however, very kindly examined material reared from grasshoppers at Wellington, Kans., and other places, with the result that the following species may now be considered parasitic, having been reared from grasshoppers: *Sarcophaga hunteri* from Payson, Utah, Wilson Creek, Wash., Hamburg, N. Y., and Wellington, Kans.; *S. sarraceniae* from Wellington, Kans., and Washington, D. C.; *S. sinuata* Meig., one specimen from Columbia Cross Roads, Pa.; *S. helicis* from Wellington, Kans.; *S. cimbicis* from Wellington, Kans.; and *S. kellyi* from Wellington, Kans., Washington, D. C., and points in New Mexico, Arizona, and Utah. It appears from the rearings that *S. kellyi* predominates, *S. sarraceniae* being second in abundance according to the material reared from grasshoppers at Wellington, Kans., and the other species occurring rarely.

## REFERENCES IN LITERATURE TO THE HABITS OF SARCOPHAGIDS

Frank Calvert (1882)[1] has reported *Sarcophaga lineata* Fall. as being destructive to locusts in the Dardanelles.

C. V. Riley (1875) has recorded *Sarcophaga carnaria* L. as having been reared from *Melanoplus spretus* Uhl. Dr. Aldrich has shown that this species of Sarcophaga does not occur in North America and that references to it in literature must be referred to another species, or most probably to several native species.

Riley also records rearing sarcophagids from the body of a mantid (Riley, 1875), several species of grasshoppers, and the cotton worm *Alabama* (*Aletia*) *argillacea* Hübn. (Riley, 1885), but was of the opinion that these flies were scavengers.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 445.

Comstock (1879) reports having reared *Sarcophaga sarraceniae* from *Alabama* (*Aletia*) *argillacea*, and gives some interesting data regarding the biology of the Sarcophagidae.

Coquillett (1892) observed sarcophagids attacking locusts in California during the summer of 1891, and, although he did not succeed in rearing the adult flies, his observations as to the method of attack agree quite closely with those of the writer.

Lugger (1896) observed a species of Sarcophaga, erroneously determined as *S. carnaria*, striking grasshoppers, but erred in stating that it deposited an elongated white egg upon the body of the locust.

S. J. Hunter (1899) has expressed the opinion that species of Sarcophaga which he observed deposited their eggs upon the soft body of the locust immediately after molting had occurred.

H. A. Morgan (1901) has published a list of Sarcophagidae reared from grasshoppers in 1900.

Lahille (1907) states that he has reared a lot of sarcophagids from grasshoppers and thus has recognized them to be parasitic. He did not, however, make any observation on the method of larviposition of the sarcophagids.

Künckel d'Herculais (1893–1905) has described the method of larviposition of *Sarcophaga clathrata* Meigen. His observations were as follows:

> We saw swarms of flies on the ground, flying in the neighborhood of the grasshoppers. We were surprised to see them introducing a tiny larva into the abdominal segments of the grasshoppers (*Stauronotus maroccanus*), but observing patiently and scrupulously they finished the attempt before we were able to seize them. (Translation.)

D'Herculais reared large numbers of *Sarcophaga clathrata* from dead grasshoppers collected in Algiers, and stated that the parasites materially reduced the numbers of the grasshoppers.

It appears from the statements made by the observers quoted herein that sarcophagids have really been known to be parasitic on grasshoppers for a number of years, and yet each of the observers, with the exception of D'Herculais and Lahille, seemed to doubt this fact, on account of the supposedly well-known carrion-feeding habits of the genus. However, the observations by Lugger, Coquillett, and C. N. Ainslie substantiate those of the writer as to the parasitic habit, and if the flies observed by Lugger and Coquillett had been collected they would very likely have proved to be *Sarcophaga kellyi*, since this species predominates in the rearings of Ainslie and the writer.

It is hoped that the facts stated herein will place these species on their correct footing as parasites and will eliminate all doubt as to their habits.

## PARASITES OF THE SARCOPHAGIDS

From the puparia of Sarcophaga were reared *Perilampus hyalinus* Say, only one specimen issuing from each puparium. This species, however, has been fully discussed by H. S. Smith (1912).

*Chalcis coloradensis* Cress. was also reared from a number of the puparia of *Sarcophaga kellyi*, only one adult issuing from each puparium. A few specimens of this species have been collected in eastern Colorado, and on July 27, 1900, Mr. George W. Martin, of Sterling, Colo., sent a number of grasshoppers to Washington which were heavily infested with sarcophagid larvæ. On August 10 adults of *Sarcophaga sarraceniae* issued, and on August 14 two secondary parasites (*Chalcis coloradensis*) issued from puparia of the Sarcophaga.

Several individuals of *Aphaereta* sp. were reared from a few puparia of *Sarcophaga kellyi* at Wellington, Kans., as many as 8 to 12 issuing from each puparium, and several *Eupteromalis* sp. were reared from puparia, the larvæ of which were collected at Dodge City, Kans., in April, 1913. About 10 to 12 adults issued on June 3 from each puparium, and every effort was made to rear them on parasitized grasshoppers, but with negative results.

The Perilampus, Chalcis, and Aphaereta issued during the late winter and early spring of 1913 from puparia in the laboratory under laboratory conditions, and no life-history work could be done on them. Continued observations on grasshoppers during the season of 1913, with special effort toward working out the method and time of oviposition and habits of these hyperparasites, were disappointing; and even a lot of sweepings of crops and weeds where grasshoppers and sarcophagids were very numerous did not reward the writer with a single specimen.

To what extent the parasites of the sarcophagids affect the efficiency of the latter could not be estimated, but it is to be hoped that they never become more than museum rarities.

## DESCRIPTION OF SARCOPHAGA KELLYI

By J. M. Aldrich

**Sarcophaga kellyi,** n. sp. (Pl. XL, fig. 1).

Male.—Front at narrowest about one-fifth as wide as the head (Pl. XL, fig. 2)—average of 10 measured with micrometer and compound microscope is 0.216 mm., the extremes being 0.195 and 0.241 mm.—brownish at vertex, becoming silvery with a slight yellow cast below, the same color extending down the orbit to the lower corner of the eye; frontal stripe dark brown, as wide as one orbit on the upper part; a single pair of vertical bristles (the normal inner pair) inserted somewhat behind the vertex proper (Pl. XL, fig. 3); ocellar bristles normal; frontals ordinary, the upper pair stout and recurved, the lowermost pair inserted toward the eye margin; side of face about half as wide as the median depression, with a few fine hairs in a row next the eye below; antennæ black, third joint about twice the second, reaching two-thirds of the way to the oral margin; arista plumose for more than half its length; vibrissæ slightly above the oral margin, the ridges above them with only a few hairs close down; beard whitish; palpi black; proboscis smallish, retracted.

Thorax (Pl. XL, fig. 1) rather narrow and long, cinereous, slightly ocherous, dorsum with 5 ill-defined subshining black stripes, of which the median one is continued narrowly on the scutellum, and the lateral are abbreviated at both ends; at the front margin another stripe, narrow but distinct, beginning on each side of the median one, but soon disappearing; four postsutural dorsocentrals, the first and second behind the suture smaller than the remaining two, and one or the other of them occasionally represented only by a large hair, but only in rare instances (in the related *Sarcophaga cimbicis* there are three postsutural dorsocentrals, but the anterior one is large and equally spaced with the others); a moderately large pair of prescutellars; acrostichals before the suture about two pairs, rather slender; scutellum with two large bristles on each side, a small apical cruciate pair (absent in two of about 200 specimens examined), and a slightly larger subdiskal pair; notopleurals four, alternating large and small; pleura concolorous with dorsum, three sternopleurals; calypters waxy white, the lower edge with silky whitish hairs; halteres brown.

Abdomen rather narrow, not much curved downward toward apex, yellowish cinereous pollinose, with 3 dorsal blackish ill-defined stripes, most distinct when viewed from behind; segments 1 to 3 with lateral macrochætæ only; segment 4 with a circle of about 16; hypopygium (Pl. XL, fig. 4) moderately prominent, its first segment black in ground color, opaque, with yellowish cinereous pollen, on its apical margin with a row of about 8 very distinct bristles, the row slightly interrupted in the middle; second segment of hypopygium red, with irregular, scattered hairs.

Forceps at base slender, wide apart, yellow, changing to black at about one-fourth the length; at about the middle there is a sudden expansion on the lower inner side (morphologically the dorsomedian), ending in a somewhat recurved point; from this the member tapers very irregularly to the tip. In profile the forceps have an evident

bend dorsad near base. There are numerous stubby, slightly recurved spines on the black part, as shown in Plate XL, figs. 4, 5, and 6. The basal hooklets of the penis are small, not shown in the illustrations; both are recurved at tip, black and approximately equal in length; the posterior has a long hair just before the point on the ventral side; the anterior is wider on the basal half and has a thumblike, sharp point projecting forward, separated from the apical point by a deep incision. The central part of the penis is black and highly chitinized dorsally, prolonged in a pair of slender, upcurved pieces which extend past the softer parts as shown in Plate XL, fig. 4; the ventral soft part consists of two deep irregular folds side by side, without distinct accessory structures or apical lobe.

Legs black; middle tibia on outer front side with two good-sized bristles near the middle; hind tibia on the inner median edge with a sparse row of 8 or 10 fine erect hairs about as long as the tibia is thick, but not villous in any sense, as in some species of Sarcophaga.

Wings hyaline, no infuscation on small cross vein; angle of fourth vein somewhat acute; third vein with a short row of about 7 hairs, first vein bare. Length, 9 to 10 mm.

FEMALE.—More grayish in general color than the male. Head (Pl. XL, fig. 7) wider than that of the male, the front at narrowest (the vertex) one-third as wide as entire head (average of 10 measured with micrometer is 0.339 mm., the extremes being 0.291 and 0.360 mm.); two stout pairs of vertical bristles; two strong orbitals; lower frontals inserted toward the eye margins as in male; palpi much stouter than in male, but ending in a point; scutellum without the small apical pair of bristles; abdomen oval, somewhat tessellated, the three stripes less distinct; fifth segment red, with a row of small bristles at apex from one-half to two-thirds as long as those of the fourth segment; no chitinous ovipositor showing in the many specimens examined. Length, 8.5 to 9.5 mm.

TYPE.—Cat. No. 18250, U. S. National Museum. A male specimen from Wellington, Kans., Webster No. 2250.

MATERIAL EXAMINED.—173 males, 138 females, Wellington, Kans. (Kelly); 62 males, 66 females, Elida, N. Mex. (H. E. Smith); 4 males, Gila River, Ariz. (R. N. Wilson, Webster No. 10535); 2 males, 7 females, Colorado (Hough Coll.); 1 male, 4 females, Wawawai, Wash., reared from grasshoppers in the fall of 1913 by M. A. Yothers, assistant entomologist of the Washington State Agricultural Experiment Station, emerged on September 2 and 13; 3 males, 4 females, Wilson Creek, Wash. (Reeves); 1 male, Payson, Utah, August 11, 1911, reared from grasshoppers (C. N. Ainslie); 13 males, 3 females (Riley's No. 733p);[1] 1 male, 1 female, marked "From *Caloptenus differentialis* Bessey.—*Sarcophaga carnaria*, 174" (referred to in Riley's Seventh Missouri Report, p. 180; reared at Ames, Iowa); 10 males, 1 female (Riley's No. 315a);[2] 2 males, Riley's No. 722p,[3] the label on the pin of one specimen reading "From eggs of *spretus*, Sept. 10, '76," on the other, "Par. on fledged *C. spretus*, sent by Wm. Cutter, Junction City, Kans.—issued Oct. 15, '76"; 4 males, Dallas, Tex., labeled, "From *C. spretus*, May '77, Boll";[4] 3 males, 1 female, Aweme, Manitoba, reared from *Melanoplus atlanis* (N. Criddle), sent by J. D. Tothill.[5]

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[1] The notes, still preserved in the Bureau of Entomology, give the following data:
*Sarcophaga sarraceniae* Riley, bred from eggs of *spretus*. Brought by Prof. C. V. Riley from Kansas, Nov. 16, '76, 3 larvæ in a tin box. One larva put in box 8–72. Also from Manhattan, Kans. Box 8–5. Jan. 22, '77. Two of the flies issued. Spread and marked 733p. Jan. 26, '77. Two more issued. There are 8 chrysalids and one larva living yet. Flies spread and marked 733p.

[2] The note on this number is as follows:
Diptera parasite in *Caloptenus spretus*. May 4, 1880. Received to-day from D. D. Sanderson, Whitney P. O., Hill Co., Tex., a box containing parasitized locusts; most of the larvæ had issued on the way and a few had transformed to puparia; the locusts are perfectly emptied of their contents so that nothing but the hard parts are left. The larvæ are milk-yellow and when stretched to their fullest length measure nearly ½inch; the surface is granulated and from each of these granules arises a very minute bristle of the color of the body; at the end on upper side of the abdomen is a deep cavity; at the outer edge, each side of the cavity, are 4 fleshy short appendages, the upper pair longest and farthest apart and the lower pair shortest and near together; inside the cavity is a pair of oval spiracles, each of which has 3 longitudinal narrow openings; the edge of the spiracles and of the openings is light brown; these spiracles are situated in the upper wall of the cavity; the anal opening on under [side] is very small and situated between two tubercle-like projections; mandibles black; mounted one larva on slide 1–44–3 and a few are placed into box 4–3; remains of the locusts are placed into box 2.

[3] The entry under 722p in the notes of the former Division of Entomology relates to anthomyiids reared from locust eggs, and could not apply to this second specimen, nor to the first except by misidentification.

[4] Mr. Boll was an agent of the Government in entomological work at that time.

[5] Since preparing this manuscript I have determined specimens of *Sarcophaga hunteri* reared by Prof. A. L. Quaintance from the codling moth, probably from larvæ (Quaintance No. 6208). Heretofore it has been known exclusively as a grasshopper parasite. This is one of the species upon which Mr. Kelly made his observations, and in the light of Prof. Quaintance's record it is evident that these flies do not always deposit their larvæ while on the wing. Therefore both Mr. Kelly's and Mr. Smith's observations (p. 437) were correct.—J. M. A.

The nearest relatives of *Sarcophaga kellyi* are *S. cimbicis* and an undescribed species. *S. cimbicis* differs in having three postsutural dorsocentrals, as above mentioned, and also in having a smaller, wholly red hypopygium; the undescribed species differs in having the rows of frontal bristles not diverging toward the eyes at the lower end (subgenus Ravinia), no small pair of apical scutellar bristles in male, and in having the abdomen in the male strongly arched downward toward the tip.

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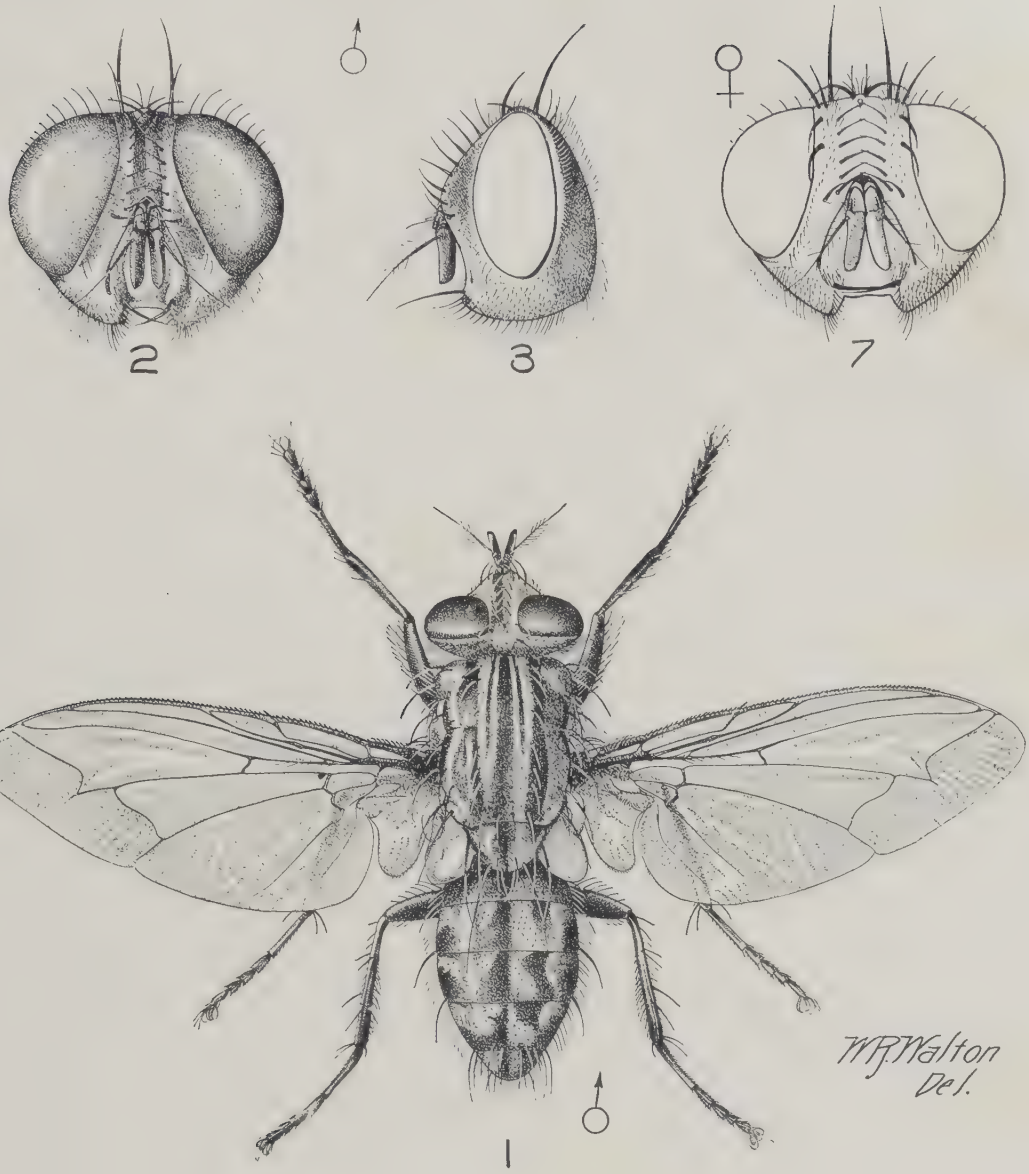
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## PLATE XL

Fig. 1.—*Sarcophaga kellyi:* Adult male fly, dorsal view. Greatly enlarged.
Fig. 2.—*Sarcophaga kellyi:* Head of male, front view.
Fig. 3.—*Sarcophaga kellyi:* Head of male, lateral view.
Fig. 4.—*Sarcophaga kellyi:* Hypopygium of male, lateral view. Greatly enlarged.
Fig. 5.—*Sarcophaga kellyi:* Left half of main forceps of same, ventrolateral view.
Fig. 6.—*Sarcophaga kellyi:* Lateral view of same.
Fig. 7.—*Sarcophaga kellyi:* Head of female, front view. Original.


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# PAPAYA FRUIT FLY

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## INTRODUCTION

The existence in Florida of a fruit fly peculiar to the papaya (*Carica papaya* L.) was brought to the attention of this department in December, 1905, by the receipt of some fruits of this plant infested with large maggots of an unknown fly. This infested fruit was forwarded by Mr. P. J. Wester, then in charge of the Subtropical Plant Introduction Field Station at Miami. From this material an adult insect was ultimately reared and was determined by the late Mr. D. W. Coquillett as *Toxotrypana curvicauda* Gerstaecker. The papaya not being a plant of special economic importance at that time, no further investigation was made, but in the summer of 1912, Mr. H. M. Russell, an assistant in the Bureau of Entomology stationed at Miami, again reported the presence of maggots in locally grown fruit. In the meantime it had developed that the papaya might reasonably be expected to become an important commercial crop in Florida, and therefore this insect at once assumed an economic importance, in that it presented a serious check to such commercial development.

Infested fruit was again obtained from the Plant Introduction Field Station at Miami through Mr. Edward Simmonds, now in charge of that station, and from this material Mr. E. R. Sasscer, of the Federal Horticultural Board, reared in November, 1912, a single female fly. In the meantime, in October, the junior author had been making field studies of the insect at Miami. Data on its larval habits were obtained, and adults were reared, both from wild and from cultivated papayas. All of this reared material, together with a captured specimen, proved to be *Toxotrypana curvicauda*. The senior author followed up this work in November, 1912, at Miami, and carried the exploration to the Florida Keys and to the Island of New Providence (Bahamas). The habits, life history, and descriptive details given below are based on these studies.

## OLDER RECORDS AND DISTRIBUTION

The specific identity of the papaya fly having been established, an examination of the material of this species and associated references from various sources in the United States National Museum developed several older records.

Probably the earliest specimens received were a set of four sent by Mr. Carlos Wercklé from the town of Santo Domingo in Costa Rica. They bear no date, but are labeled "In fruit of Carica," and were determined by Mr. Coquillett. Under date of July 21, 1910, Dr. P. Osterhout sent the fly from Bocas del Toro, Panama, with the information that it did serious damage there to the fruit of papaya. During September, October, and November, 1910, the fly was reared from larvæ infesting the papaya

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in Porto Rico, both by Mr. W. V. Tower and by the late Mr. C. W. Hooker, but these specimens were not received for identification until much later. Quite recently a brief account by Hooker (1913)[1] of the occurrence of the papaya fruit fly in Porto Rico and its infestation of the "lechosa," as the papaya is there called, has appeared.

In addition, the fly has been reported, without indication of its habits, from the following localities: Island of St. Jean in the Danish West Indies (Gerstaecker, 1860), erroneously given by Van der Wulp (1898) and Aldrich (1905, p. 600) as "St. John, Antigua"; Brazil (Bigot, 1884); Pebas, Peru (von Röder, 1891); Yucatan (Snow, 1895; Van der Wulp, 1898). It is evident that *Toxotrypana curvicauda* has a very wide distribution in tropical America, probably coextensive with its food plant.[2]

In Florida, Miami appeared to be the northernmost point of distribution at the close of 1912. Examination of cultivated, wild, and semiwild fruit at Little River and in the hammock belt as far north as Arch Creek showed no trace of the larvæ. Recently, in 1914, however, the fly has been reported as very destructive at Palm Beach, considerably farther north. To the south it was found at Key Largo in 1912. Here papayas grow scattered about in cleared land, and the fruit was found infested to a considerable extent. Mature larvæ were obtained from ripe fruit and the imago reared therefrom. Under papaya plants that had been fruiting, puparia or empty pupal shells could always be found. At Marathon, about midway on the Florida Keys, no traces of the fly could be found, although many wild papayas occurred among the wild growth. Conditions farther to the south appeared to be unpromising, and no further search for the fly was made in that direction.

Considerable interest attached to the question whether the papaya fruit fly existed in the near-by Bahama Islands, as it seemed highly probable that it was from there that the fly had found its way to Florida. This point was definitely decided. The Island of New Providence, which lies about 200 miles east of Miami and which is in most frequent communication with the mainland, was visited and the papaya fruit fly located without difficulty. Adults were reared from some of the larvæ and puparia obtained on this island, which precludes all doubt as to the identity of the pest.

## DESCRIPTION OF PAPAYA FRUIT FLY

### THE ADULT

The papaya fruit fly (*Toxotrypana curvicauda*) belongs to the dipterous family Trypetidae and exhibits a certain superficial resemblance to a common brown wasp (Polistes). This is due not only to its similarity of size, form, and general coloration, but in life this is accentuated by the manner in which it walks about on the fruit, with its body well elevated upon its slender legs, and by a certain nervousness of movement. The female is remarkable for its long and slender curved ovipositor, which exceeds the length of its body.

FEMALE (Pl. XLI, fig. 1).—Yellow and brown, marked with black. Head broad, fully as wide as the thorax at wing base, inserted upon a slender prothoracic neck; eyes elongate, subovate, prominent, bulging at the sides, separated by about their own

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 453.

[2] The papaya is a native of tropical America and is now cultivated for its fruit throughout the Tropics of both hemispheres. Its introduction into Florida is probably comparatively recent, although no data bearing on this point are available, and the wild plants in southern Florida and the adjacent keys are seedlings from such introduced plants.

width; occiput, frons, face, and cheeks yellow, a black transverse patch at the ocellar triangle, a diffuse smoky patch over insertion of antennæ and another along oral margin, and a black spot crossing cheeks from lowest part of eye; antennæ piceous or dull ferruginous, the third joint elongate, rounded at apex, the arista inserted close to its base, nearly twice as long as the joint, very slender, with very fine ciliation; proboscis and palpi black. Thorax convex, broadest at wing base, narrowed anteriorly, yellow, with a ferruginous shade on the disk, the mesonotum with several pairs of abbreviated blackish stripes and a transverse spot in front of the scutellum, the pleuræ with three irregular, transverse, blackish bars; scutellum yellow, with sharp black lateral angles; postnotum ferruginous and black. Abdomen pedunculate, with six well-defined segments, of which the second is much the longest; venter channeled at the sides; colors yellow and dull brown, with dark diffuse bands at apex of first, middle of second, and bases of the following segments; ovipositor longer than the body, slender, cylindrical, strongly curved, thickened basally, ferruginous. Legs slender, rather long; femora yellow, ferruginous at bases and apices and with a dark oblique band near middle; tibiæ yellow and ferruginous, the anterior pair darker; tarsi ferruginous, shading to brown, densely pubescent. Wings long, rather narrow, rounded at apex, all the cells much elongated; second vein with an angulation or loop opposite the end of the first vein and usually just beyond this with a slighter one, which in many specimens sends out at right angles a spur toward the costa; last section of the fourth vein sinuate; lower end of posterior cross vein close to wing margin; anal cell with the lower angle drawn out very long and narrowly; colors hyaline and ferruginous yellow, the yellowish color involving the anterior region to the second basal cell and the anterior portions of the first basal and first posterior cells, as well as the anal and the base of the third posterior cell. Length: Body, exclusive of ovipositor, 8.5 to 12 mm.; ovipositor (measured in a straight line from base to tip) 9 to 14 mm.; wing, 8.5 to 12.5 mm.

MALE (Pl. XLI, fig. 2).—Closely resembles the female in coloration and general appearance, without the ovipositor. The abdomen is pedunculate and blunt at the tip, less distinctly banded than in the female and more hairy; the frons is slightly wider; the spur of the second vein usually joins the costa, and shortly before this there is often in addition a short spur projecting into the submarginal cell. Length: Body, 11 to 13.5 mm.; wing, 8.5 to 11 mm.

## THE EGG

The eggs (Pl. XLII, fig. 1) are of very unusual shape. They are very long and slender, fusiform in the micropylar half, the opposite half drawn out into a cylindrical stalk. The length of the entire egg is from 2.55 to 2.75 mm., the greatest diameter 0.18 to 0.2 mm., the diameter of the attenuated portion 0.06 to 0.07 mm. The surface is smooth, without reticulation or sculpture.

The eggs were procured from gravid females by dissection. The number of eggs produced by a single female appears to be slightly in excess of 100; the counts from two females, both showing a distended abdomen and probably containing a nearly full complement of eggs, gave 103 fully developed eggs in each case. No eggs in process of development were present, which indicates that all the eggs are disposed of within a short period.

## THE LARVA

The larvæ (Pl. XLII, fig. 2) are shining, dirty greenish white in color while feeding upon the interior seed mass. Larvæ that have matured within the ripened fruit and that have penetrated into the meat are the same rich golden-yellow color as the ripe fruit.

Form moderately stout, subcylindrical, tapered anteriorly to the mouth, highly polished; head segment closely transversely striate beneath, mouth hooks blunt, well separated; anterior spiracles with about 25 lobes; body segments nearly smooth above, ventrally with a transverse callosity at the anterior margin, beyond the third segment also with a callosity at the posterior margin, the surface of these callosities with numerous, irregular, transverse, raised, punctate striæ, segments beyond the third also with a deep median transverse impression; posterior end truncate, the stigmatal area slightly depressed, surrounded by a groove; stigmatal plates small, separated by about their own width, each with three transverse, straight slits, slightly diverging outwardly; anal tubercles small, upon a swelling bordered by two callosities.

### THE PUPARIUM

Puparium (Pl. XLII, fig. 3) of the usual stout subcylindrical form, with rounded ends; slightly depressed ventrally, the larval callosities obsolete; laterally with a series of shallow longitudinal impressions; stigmatal area hardly differentiated; anal papillæ prominent; color ferruginous yellow to light ferruginous.

### HABITS OF THE LARVÆ

The papaya fruit-fly larvæ occur in the interior of the fruit, first feeding in the central seed mass, but later, as they mature and the fruit ripens, working into the meat and ruining the fruit (Pl. XLII, fig. 4). The number of larvæ in a single fruit varies from 2 or 3 to 20 or more. Sometimes larvæ of different sizes occur within the same fruit, showing that the infestation was from more than one oviposition.

The cultivated fruit in the Subtropical Field Station at Miami was by no means so generally infested as the wild fruit in the hammock south of it. Evidently infestation stood in relation to the thickness of the meat of the fruit, in that cultivated forms having the thickest meat were least affected. Several of the latter, some still small and others nearly ripe, were cut open, and no maggots could be found. The only evidences of injury to these thick-meated fruits were numerous scars that had the appearance of egg punctures. On the other hand, the fruit of volunteer plants in the Field Station, with thin meat and large seed cavity, was frequently infested.

The wild fruit in the hammock[1] was found to be very badly infested with papaya fruit-fly larvæ. This wild fruit is very small and seldom exceeds 2 inches in diameter. The meat is thin, and there is comparatively little flow of juice when the surface is injured.

To determine the amount of infestation in the wild fruits of different sizes or ages, an examination was made of small fruits about three-fourths of an inch in diameter, medium-sized fruits, and large ripe fruits. Out of 208 small fruits, 41 showed infestation, and 167, or 80 per cent, were sound. Out of 52 medium-sized fruits 26, or 50 per cent, were free from infestation. Examination of 25 nearly ripe fruits showed that none were sound. Again, in a miscellaneous lot of 63 fruits, 32, or over 50 per cent, were infested. In general, small or young fruit is much less infested than the older fruit, the flies evidently selecting the larger and more mature fruits for oviposition.

A remarkable fact was the occurrence of dead full-grown larvæ in fruits externally sound. In the 208 small fruits, 33 living and 3 dead larvæ were found. In the 52 medium-sized fruits there were 22 living larvæ and 1 dead larva. In the miscellaneous lot of 63 fruits there were 82 living larvæ and 38 dead ones. In the 25 nearly ripe fruits, however, the dead larvæ exceeded the living ones, there being 12 of the former and 6 of the latter. The importance of these data will be discussed farther on.

It was found that contact with the juice of the unripe fruit is quickly fatal to the larvæ, which explains the presence of the dead larvæ in

[1] In the hammock, the virgin tropical vegetation to the south of Miami, papaya plants were growing adventitiously in large numbers, particularly along the edges of the hammock and in the more open spots. The abundance of these wild papayas was traceable to the operations of real-estate men, who, about a year earlier, cleared the trees and shrubs from a portion of the hammock to lay out streets. In these cleared places the papaya had sprung up in great profusion. On the authority of Mr. W. E. Safford, of the Bureau of Plant Industry, wherever the papaya is grown wild plants very quickly spring up adventitiously in the vicinity, particularly in newly cleared land. Undoubtedly the seeds are distributed through the agency of birds, and in this way the plants make their appearance at a distance from the point of cultivation.

unripe fruit. These dead larvæ are always full grown and therefore perished at the time that they would have normally made their escape from the fruit, in order to pupate in the ground. In ripe fruit mature larvæ are easily able to bore out through the soft meat, which then no longer exudes the gummy juice characteristic of unripe fruit; but the sticky exudation of the flesh and rind of unripe fruit is fatal to the larvæ, probably by asphyxiation.

## PUPAL PERIOD

The larvæ, when about to pupate, usually leave the fruit as just described and fall to the ground. The pupal period is passed either under some fragment of coral rock or in the soil at a depth of 1 or, at the most, 2 inches below the surface. Rarely puparia were found within the hanging fruit. The pupal period varies according to the meteorological conditions. In Porto Rico, Hooker (1913), found that the duration of the pupal period was from 17 to 21 days. The observations of the authors, made in the cool season of the year, showed a pupal period of from 30 to 42 days. They further show that moisture conditions, as well as those of temperature, affect the length of the pupal period, and that excessive dryness has a retarding effect and if prolonged may prove fatal.

## HABITS OF THE ADULT FLY AND OVIPOSITION

Special effort was made to learn something of the habits of the adult fly. The senior author, while at Miami, made frequent visits to the papaya plantation at the Subtropical Plant Introduction Field Station and to the hammock south of it, with this object in view. Careful watching in the vicinity of the papaya plants, and vigorous searches were alike unsuccessful until near the close of the investigation. It was then found that the flies are only active for a very short period just before sunset.

About half an hour before sunset a female papaya fruit fly came with rapid flight and alighted unhesitatingly upon a well-developed but green papaya. After walking about a little on the top of the fruit, the fly began to insert its long ovipositor, and in a remarkably short time had sunk it full length into the fruit. As soon as the fruit was punctured the milklike liquid, which the unripe fruit exudes wherever injured, welled forth and began to trickle down the side. The fly very soon withdrew its ovipositor and was about to take wing when it was captured. In the course of about 15 minutes some eight or more flies were seen, all behaving in the manner described, and four of these were captured in the act of puncturing the fruit. The next evening, in the same place, a few more female flies were seen at about the same time, but none earlier and none later. It seems evident that flight and oviposition occur at a definite time in the evening, a little before sunset, and are governed by the amount of light. Under the conditions observed, viz, on bright, nearly cloudless days, this activity does not last more than 15 or 20 minutes. There may also be a morning flight, for Mr. Simmonds stated that he had captured the fly early in the morning.

It is evident that the female endeavors to thrust her ovipositor through the meat of the fruit to deposit her eggs in the central seed chamber, and it is only in the varieties with thinner meat that she can succeed. With the exception of mature larvæ in ripe fruit, the larvæ are always found within the seed mass. It is only when they are full grown, or

nearly so, that they enter the meat, work their way out, and drop to the ground, in order to pupate just beneath the soil. As already pointed out, the juice from the injured meat of the unripe fruit is fatal to the larvæ; therefore the eggs must be placed beyond it in the seed chamber.

Later observations were made by Mr. C. A. Mosier, of Little River, Fla., who has had many opportunities to observe the papaya fruit fly. He recently forwarded the following notes on its habits:

> The flies do not oviposit on very young fruit, but are found more on large to full-grown fruit. One female was captured with the ovipositor fully embedded in three-fourths ripe fruit. I also noted that the flies skipped deformed and gnarled fruit. More females are out on dark cloudy days, while the males are active and predominate on warm sunny days and are sluggish on dull days.
>
> Also, I have found three females dead, or nearly so, that had been trapped by the juice of the fruit coagulating before the ovipositor was withdrawn.

## PAPAYA FRUIT FLY RESTRICTED TO THE PAPAYA

The fact that in a fruit-growing section like southern Florida no complaints of infestation of fruits other than the papaya had been made was in itself evidence that the pest is peculiar to this fruit. This was confirmed by examination of other fruits and by transfer experiments. Immature larvæ transferred to citrous fruits failed to complete their transformation and died.

## RAPID INCREASE OF THE FRUIT FLY

During the last two years, more particularly as a result of the increased cultivation of the papaya in southern Florida, this fruit fly has also rapidly increased and extended its range so as to seriously threaten the future development of the papaya industry. Some varieties of Philippine stock producing large fruit grown at Lemon City, Fla., are apparently free from attack. A very fine large variety of fruit grown at Lemon City, Fla., from Philippine stock was found to be free from the larvæ.

## CONTROL

It has been pointed out that fruit with very thick meat escapes infestation. While the papaya fruit fly attempts to oviposit on such fruit, the thickness of the meat prevents the tip of the ovipositor from reaching the seed cavity, and in the meat itself the larvæ can not live. It was further found that in some fruits the larvæ had reached maturity before these had ripened and had been killed by the sticky juice of the green fruit in endeavoring to escape. The means of control that now seem valuable are the production of varieties of papaya that have thick meat and that ripen slowly, and the conscientious destruction of adventitious or wild papaya plants and of all infested fruits. All plants with inferior fruit should be eliminated.

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## PLATE XLI



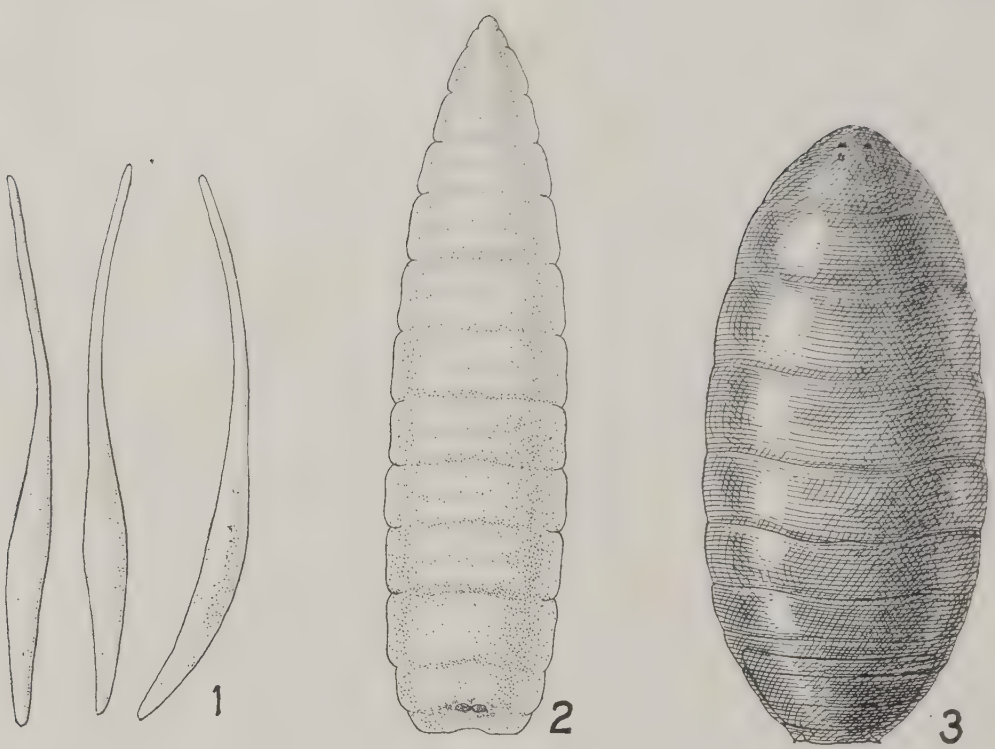
Fig. 1.—*Toxotrypana curvicauda* Gerst.: Female. Enlarged.
Fig. 2.—*Toxotrypana curvicauda* Gerst.: Male. Enlarged.



1



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## PLATE XLII

Fig. 1.—*Toxotrypana curvicauda* Gerst.: Eggs. Enlarged.
Fig. 2.—*Toxotrypana curvicauda* Gerst.: Larva. Enlarged.
Fig. 3.—*Toxotrypana curvicauda* Gerst.: Puparium. Enlarged.
Fig. 4.—Papaya fruit infested with larvæ of *Toxotrypana curvicauda* Gerst. Somewhat reduced.

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